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SPINAL CORD REGENERATION IN THE RAT*

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(Received for publication December 20, 1938)

INTRODUCTION

REGENERATION in the central nervous system of non-mammalian vertebrates is generally accepted (Lorente de Nó, 1921; Pearcy and Koppanyi, 1924; Detweiler, 1926; Hooker, 1932; Tuge and Hanzowa, 1937), but in mammals, regeneration is said not to occur or to be abortive (Lee, 1928; Cajal, 1928; Spatz, 1930; Rossi and Gastaldi, 1935). The more important factors in this non-regeneration are held to be (i) the absence of "embryonic" cells to help restore the gray matter (Spatz, 1930; Carlson, 1924), (ii) the lack of Schwann cells (Spatz, 1930; Yamada, 1906–8) and the lack of neurotropism and growth pathways (Cajal, 1928 for discussion). The more recent work on mammalian central nervous system regeneration has been largely concerned with rat foetuses—which should have capabilities for regeneration between those of the lower vertebrates and of the adult mammal. Gerard and Koppanyi (1926) and Gerard and Grinker (1931) transected foetal rats in utero or just after birth. They found considerable return of function, but could present little certain evidence of anatomical restitution. Completely negative anatomical findings were reported by Hooker and Nicholas (1927, 1930) and Nicholas and Hooker (1928) after transection with the cautery, but they did find much return of function, attributed to the transmission of impulses by collateral pathways and to mechanical pulling of tissues to initiate reflexes past the cut. About this time, however, Migliavacca (1930a, b) reported two cases of anatomical and physiological regeneration in foetal and newborn rats. In their review of mammalian regeneration, Rossi and Gastaldi (1935) attributed this anatomical regeneration to regrowth of spinal roots and to possible incomplete section of the cord, leaving the return of physiological function to be explained by the collateral transmission of Hooker and Nicholas.

Only one paper on regeneration in young or adult mammals has appeared since 1928 (for reviews up to this time see Lee, 1928, and Cajal, 1928). Marburg (1936) found no regeneration in dogs with one or both lateral columns cut. The bulk of work has been done on rabbits, cats, and dogs. It had been noted by Gerard and Koppanyi (1926) that spinal cord section in young adult rats was invariably followed by bladder disturbances, edema, and early death. Ssamarin (1926) abandoned the use of rats in his spinal cord studies "da diese Tiere die Schnitte nicht vertrugen und zugrunde gingen" (p. 374). The present work was undertaken to maintain, if possible,

* The present investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

chronic adult spinal rats, to elucidate the factors involved in non-regeneration of adult mammalian spinal cord, and to attempt to activate the abortive processes of regeneration.

The distribution of the anterior spinal artery of the rat (the chief source of blood for the spinal cord in this animal) was first investigated so that a level of section could be chosen which would avoid anemia of the several stumps (the effects of spinal anemia are well known—Gildea and Cobb, 1930; Tureen, 1936, 1938). It is probable that the "isolation dystrophy" of the caudal stump in spinal monkeys (Sherrington, 1906) is largely due to disturbances in vascular supply.

METHODS

A. For study of the anterior spinal artery, adult rats of both sexes, submerged in warm water, were perfused through the heart with warm 10 per cent gelatin colored with trypan blue (plus 1 per cent thymol to prevent bacterial action) after the blood had been washed out with 25 cc. of Ringer. The entire body was then left over-night under cold running water or in an icebox, until the gelatin hardened. The cord was exposed from the ventral side, and the distribution of the artery and its tributaries noted.

B. Spinal cord section was performed on young (3–5 weeks) albino and hooded rats of both sexes. Under ether anesthesia, the animals were placed belly down, tied by the fore-legs only, and the skin on the dorsum extensively shaved and cleaned with alcohol. A midline incision was made from the middle of the scapulae to the last thoracic vertebra; bleeding from the infrascapular fatty tissue stopped with hot moist cotton, the laminae of two or three vertebrae removed, and, after further haemostasis, the cord severed with a fine scalpel (Bard Parker No. 11) or fine-pointed scissors. In the early experiments, completeness of the cut was insured by drawing the knife blade through the cut a second time, feeling the bony vertebral canal on all unexposed sides. Later it was found that tension on the tail at the time of sectioning favored a complete cut with one knife stroke and, since the cut ends separated 1 to 3 mm., left no uncertainty of the completeness of section. After hemostasis, either the ends were brought back into apposition or other tissues placed in the gap. In one series, bits of dorsal musculature were inserted (series RSR); in another, mashed or intact embryonic brain or spinal cord (series RT); in a third, segments of degenerated sciatic nerve (series RNC). The muscles, dorsal fascia and skin were then sewn in separate layers with silk. No dressings were applied. In a subsequent series of spinal rats (suggested by Dr. Lorente de Nó) partial sections were deliberately made in order to eliminate any injury to spinal roots. Lesions ranged from small stab wounds in the dorsal columns to lateral hemisection, at levels selected to avoid dorsal roots.

The embryonic nervous tissue was obtained from 3–4 cm. rat foetuses, removed from the mother (ether anesthesia) after the spinal cord of the recipient was exposed, and exhibiting good reflexes at this time. To obtain brain tissue, the head was cut off and the cranial contents squeezed out. More intact cord was obtained by cutting it from the spinal column with sharp scissors. The sciatics were taken from young adult rats, 2 to 4 weeks after section at the exit from the greater sciatic foramen. Adequate *asepsis* was attained by soaking instruments and skin in 70 per cent alcohol, since no primary wound infection occurred. It is essential that bleeding be well stopped before the wound is closed. The blood lost is usually quite small, but a post-operative intraperitoneal injection of 5 cc. of warmed saline was regularly given.

Operated animals were kept in solid floor cages on sawdust, since the paralyzed extremities suffer injury in open wire meshes. For 10 to 15 days, until the bladder became automatic, urine was expressed three times daily by pressure on the lower abdomen. The animals were frequently tested for sensation and reflexes and their spontaneous movements and activities observed. Finally, under ether anesthesia, the brain was exposed and stimulated (Harvard inductorium, 3 volts in the primary, secondary at 7 to 9 cm.). The animal was then decerebrated, the anesthetic discontinued, and the cerebral peduncles stimulated directly. Finally, the animals were perfused through the heart (intestines clamped off) with 20 cc. of Ringer followed by 100 cc. of a mixture (Bodian, 1937), of 80

per cent alcohol (90 cc.), 100 per cent formol (5 cc.), and glacial acetic acid (5 cc.).

The spinal cord was then exposed from the ventral surface and the region of section, including 3 to 4 segments on either side, placed in the same fixative for 24 to 48 hours, dehydrated with cedarwood oil, and embedded in paraffin. In a few instances, when the cord ends showed poor continuity or were attached to the periosteum, the cord and surrounding bone were removed, fixed, and decalcified in 5 per cent nitric acid in 70 per cent alcohol for 6–9 days. Sections were cut 20 to 25 micra thick in either frontal or sagittal planes, and mounted serially for staining with protargol by the silver-on-the-slide method of Bodian (1936, 1937). Every 10th or 12th section, however, was mounted serially on a separate slide for staining with toluidin blue and erythrosin or with Ehrlich's hematoxylin and eosin. In some cases, formalin fixation was used and blocks of cord above and below the lesion were sectioned transversely and stained as above or with an iron-haematoxylin method for myelin sheaths (Mahon, 1937). Animals dying before termination of the experiment were discarded.

Results

General observations

Blood supply. In the rat, the anterior spinal artery does not arise from the vertebrals, but rather is formed by the confluence of branches of a number of vessels ascending along dorsal roots (Fig. 1). From C1 to T12 it is built from caudal and cranial branches of 6 to 10 such arteries, irregularly distributed, and usually coming more from the left than the right. At T12, T13, or L1, a large entering vessel anastomoses through a fine connection with the thoracic portion of the artery, then curves caudally to become the lumbo-sacral portion. (In 5 males and 1 female, this vessel arose from the right; in 3 females, from the left.) Usually no vessels enter the artery below this large one. The anterior spinal artery joins the usual branches of the vertebral arteries at its cranial end; caudally it loses itself in terminal twigs.

The cord was cut above T12, to avoid the large blood vessel. Section below T13 uniformly led to degeneration of the caudal stump of the cord with atrophy of the hind-limbs, and failure to regain bladder control. (In the dog, bladder automaticity has been held to return in the absence of the lumbar cord, Goltz and Ewald,

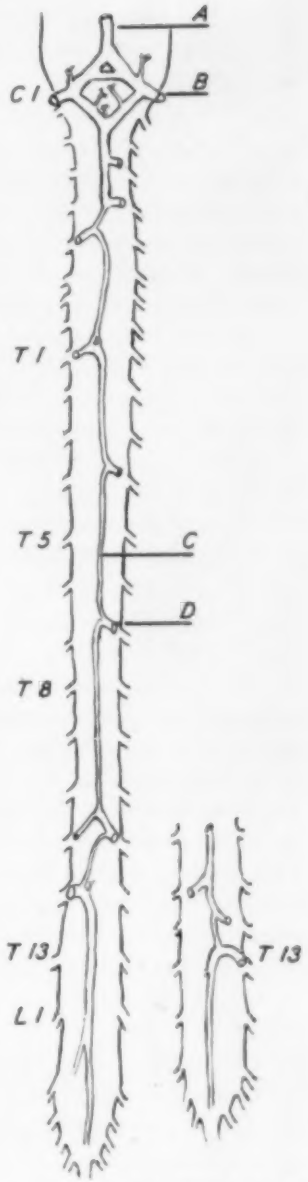

FIG. 1. Diagram of the anterior spinal artery of the albino rat, based on 5 males and 4 females: the most common variation, found in 3 of the females is given in the inset. A. basilar artery; B. vertebral artery; C. anterior spinal artery; D. radicular branch of spinal ramus of intercostal artery.

1896; but this has recently been denied, Morvan, 1936). Section above T5 interferes with fore-limb and respiratory movements. In 23 of 28 fully studied cases the section was between T6 and T9. There is no correlation between the level of cut and bladder control or length of survival.

Bladder control. In females, urine is easily evacuated by squeezing the abdomen. In males, the tenacity of the internal sphincter and the occasional occlusion of the urethral orifice with sawdust or semi-solid secretions makes necessary considerable pressure; enough to rupture the bladder in extreme cases.* Expression of urine 3 times daily provided adequate emptying and prevented cystitis, pyelitis and hydronephrosis. One emptying a day was wholly inadequate; two might suffice when evenly spaced. The quantity of urine obtained at one time was roughly recorded as: +++, 2 to 3 cc., the usual accumulation of 8 hours; +, 0.5 cc. or less; and 0. Occasionally as much as 5 cc. were expressed.

The bladder became automatic after 8 to 12 days (most commonly 9) in both sexes alike, unless degeneration of the caudal cord segment prevented any return or intercurrent illness delayed it three weeks or more. Automaticity was considered present when the three urine amounts expressed in a day totaled 5+ or less instead of the usual 9+. Well-formed fecal pellets were regularly dropped at least by the second day, so intestinal function offered no nursing problem.

With adequate bladder emptying, the gangrene and edema previously encountered (Gerard and Koppanyi, 1926) were practically eliminated. The coincidence of bladder dysfunction and edema is well shown in rat RNC 1 in which bladder control was present from the 10th day after operation. Five months later the left leg became edematous and the bladder was found to be distended and the penis engorged due to a caseous plug in the urethra. This was squeezed out and urine regularly expressed for a week, after which automaticity was reestablished and the edema entirely gone. As a further check, in four animals the postoperative bladder evacuation was omitted. All developed edema and gangrene and three died within a week.

Of 47 rats operated on (16 males and 31 females), 15 died within two weeks from bladder disturbances, etc. The rate for males was much higher for this period only. Late deaths were mostly from pneumonia. Once past the early hazards, the life of the operated rats is potentially the normal span, and the animals grow at a normal rate. Those animals living 14 days or less have not been examined in detail. Usually they died at night and the spinal cords could not be salvaged.

Reflexes. The reflex activity of spinal rats is considerable. Such animals make excellent demonstration material. An ipsilateral flexion reflex can be elicited within 1 min. after the animal awakens from anesthesia, long before proper coordination of the anterior part of the body has been regained, and consists of a simple withdrawal upon pinching the foot unaccompanied

* This is not necessarily fatal.

by after-discharge or spread. Withdrawal of the tail in response to pinching it appears on the second or third day after operation. Later (3 to 7 days) the same stimulus also causes stepping—bilateral alternate rhythmic hip and leg movements—which eventually considerably outlast the stimulus. Crossed responses are added to the uncrossed also on the 2nd or 3rd day. Pinching the toes causes ipsilateral flexion and contralateral rhythmic "pushing-away" movements, similar to the stepping response, but directed towards the stimulated foot. This defensive reaction becomes more and more marked and in later stages appears almost voluntary. In a few cases, it could also be elicited by traction on the crossed leg. Simple crossed extension appeared only in isolated instances. A complicated reaction appeared in a number of cases, about 10 days after the operation, when the animal was lifted by the chest. The caudal segments and hind legs were stiffly extended, with toes flexed, for a few seconds and then the body relaxed or "curled', with flexed toes, hip, and knee, and adducted thigh, so that the legs were closely pressed to the abdomen. This defensive shrinking position was sometimes assumed without the initial "standing" posture.

During the first week or ten days, the animal drags itself around with fore-limbs and the flaccid hind-legs are passively stretched behind or flexed as the body turns. Later, however, walking movements of the hind-legs, mainly alternate flexion of the hips, appear irregularly, usually after the legs have been dragged passively for some distance. Scratch reflexes develop between the 11th and 14th days. A standard undirected response can readily be obtained by stroking the lower abdomen or a number of other regions but never involves the leg across the midline from the stimulated skin. A mass reflex is but rarely provoked by pinching the toes and never by other forms of stimulation (*e.g.*, stroking the abdomen). "Spontaneous" movements, stepping when the animal is lifted and scratching with no apparent cause, are occasionally seen.

Physiological recovery

The reflex activity of the hind-quarters described above represents the final equilibrium of the true spinal animal. No sensation returns. Thirteen animals acquired additional motor and sensory capacity, associated with anatomical restitution. A number of tests were used.

When placed on a vertical wire mesh, spinal animals climb or, usually, descend by means of the fore-legs. The hind-legs drag passively, are not placed, and usually catch in the wire and make the rat fall. When suspended by the tail, the hind-legs, in the spinal rat, hang passively with lax toes and do not move; while in the normal, the legs and toes make searching movements and, if a support is encountered, grasp it and right the animal. When placed on its back, only the fore-quarters of the spinal animal perform righting movements and the inert rear often prevents a successful turning over. On the sensory side, the forward transmission of pain impulses (pinching tail) can be signalled by struggling of the fore-quarters or vocalization—

never observed in the spinal animal. Hind-legs remain where they fall or have been put, indicating absence of proprioception. Finally, electrical stimulation of the peduncles (and rarely of the cerebrum) of intact animals evokes generalized contractions; but in spinal ones all response stops completely at the transection level. Even with much stronger stimuli (secondary at 3 cm.) no "spread" beyond the lesion occurs and, during stimulation, the vigorously contracted front half adjoins the flaccid motionless rear.

The animals showing recovery performed somewhere between the normal and the spinal rats on these tests. In walking, toe movements contributed to the hind-leg action; and toe flexion and extension sometimes occurred while the animal lay at rest. A peculiar hopping of both hind-legs alternated with stepping movements in three rats and enabled them to progress indefinitely. Placed on a vertical mesh, eight animals used one or both hind-legs actively in climbing, even at times in descending, with seeking and some placing movements. These appeared during the second post-operative month. After the 4th or 5th week, when any of the recovering animals was hung by the tail, the hind-legs were held stiffly outstretched except for occasional rhythmic and alternating seeking movements. Effective grasping never was observed. In one animal, the left hind-leg participated in righting movements; and it readily regained its feet when placed on its back or either flank. Pinching the hind-legs or tail evoked struggling movements of the fore-quarters but no squealing. The hind-legs and toes were placed with more or less accuracy in seemingly voluntary movements and were drawn into a proper posture when displaced, indicating active proprioception. Finally, stimulation of the peduncles with moderate shocks (secondary at 7 cm.) caused movements caudal to the transection. Simple flexion or extension and rhythmic stepping or thrusting were elicited on either or both sides depending on the rat and on the exact site of stimulation. Sometimes a marked after-discharge persisted behind, but not in front of, the plane of section, so that the fore-quarters had relaxed at the end of stimulation while a hind-leg, for example, continued to step for 5 to 10 seconds.

Anatomical findings

Three rats of a preliminary series showed incompletely sectioned cords. These are satisfactory controls, for in all of them some voluntary movement was present at least by the second post-operative day and coordinated movement and sensation rapidly improved until nearly or quite normal. Similar rapid and complete recovery occurred in the later series of animals with hemisections or lesser lesions. In all other animals the cord had been completely severed and a connective tissue scar bridged the cut ends.

Cysts were often present, one on either side of the central scar, particularly in cases in which a tissue had been placed in the cut. They sometimes had thick walls containing normal nerve cells, which thus were close (in one case within 175 micra) to the scarred region (Fig. 2). In the scar were many infiltrating leukocytes and connective tissue bundles, which usually ran

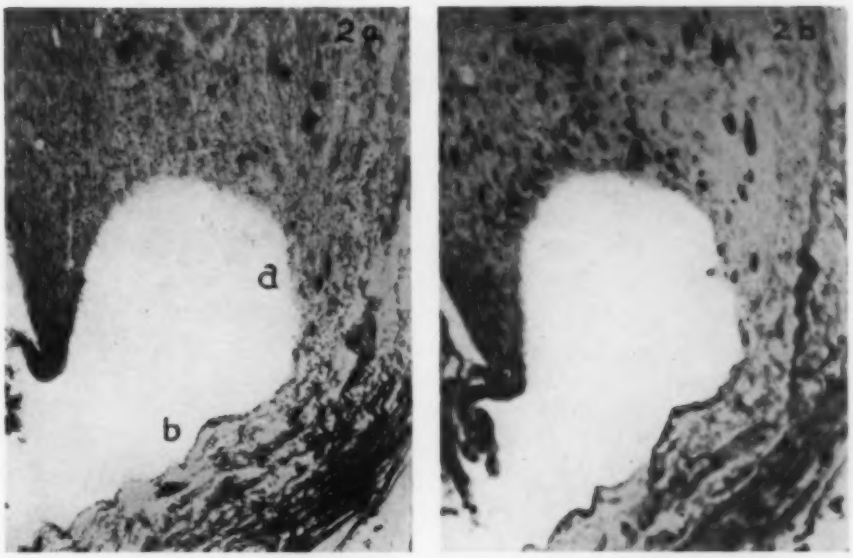

FIG. 2. Fig. 2 through 9b are photomicrographs of sagittal sections of spinal cords of operated rats. Fig. 2b and 3 are from sections stained with toluidin blue and erythrosin: the remaining are from sections stained with the silver-on-slide method of Bodian. *2a* Craniad stump of spinal cord. The dorsal half of the cut region was occupied by a cyst; *a*, clump of normal cells; *b*. connective tissue scar with new fibers arising from ventral grey matter; ×80. *2b*. Section adjacent to that of Fig. 2a. Note deeply-staining Nissl substance in cells around the rim of the cystic area: note also apparent absence of nerve fibers; ×80.

transversely across the base of the stumps or the cysts. In general, the scarring and the degeneration changes in the white matter were as described by Cajal (1928), with retraction balls, autotomy of fiber ends, axon varicosities, and the like. No extensive red cell collections, or traces of their breakdown and phagocytosis could be found.

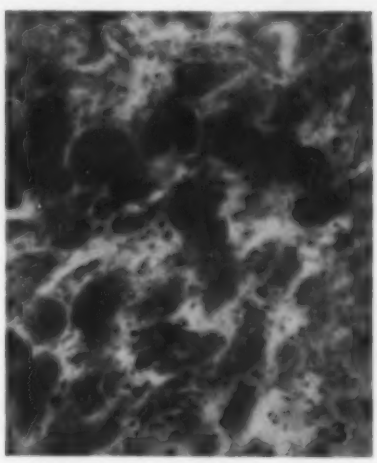

FIG. 3. Scar region with acini-like cell clusters arising from transplanted embryonic brain. Note profuse neighboring connective tissue cell nuclei; ×400.

Transplanted embryonic material in most cases did not stay between the cut ends of the cord. When it did, it either formed a dense degenerated mass or, more typically, became organized into cell clusters resembling glandular acini (Fig. 3). These "acini" were seen only following embryonic nervous tissue implantation, except in rats with partial lesions. Three of these animals showed entirely similar cell nests derived, presumably, from ependymal elements. In two cases, well differentiated pyramidal neurones developed from the embryonic implant. These small pyramids were seen in the scar

tissue, well separated from either cut stump, and their processes could be traced some distance through the scar. These neurones were quite distinct from normal cord elements and definitely developed beyond the embryonic stage at implantation.

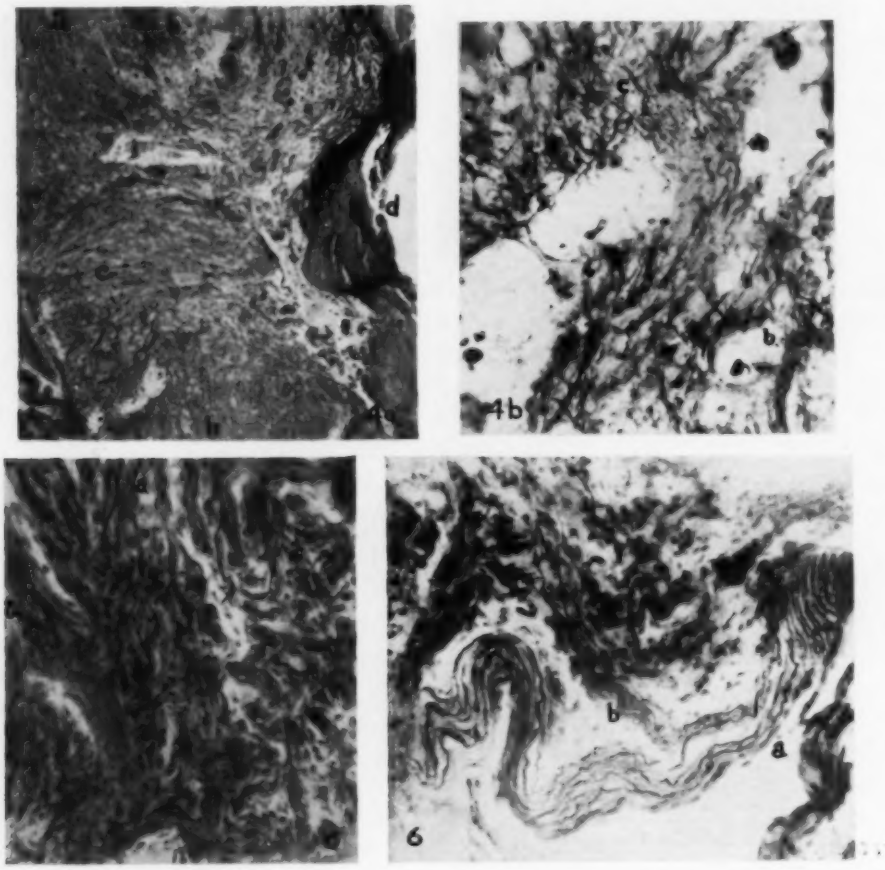

FIG. 4a. Scarred region of spinal cord, showing horizontal orientation of complete scar as compared to vertical white columns. Note nerve fibers and many *boules d'arrêt* in both stumps; ×48; *a*, upper stump; *b*, lower stump; *c*, scarred region; *d*, residual hemorrhage.

FIG. 4b. Scarred region containing haphazardly running fibers arising from neighboring stump. High power of a small region of Fig. 4a: lettering as above; ×240.

FIG. 5. Scar region containing degenerated sciatic nerve transplant; ×240; *a*, bundles of new fibers running in old nerve paths. Arrow points to a new fiber curving because of connective tissue obstacle.

FIG. 6. Origin of new fibers from ventral spinal root; ×240; *a*, ventral root; *b*, new fibers growing into scarred region.

Neither nerve cell mitoses nor any other signs of grey matter regeneration were seen. On the other hand, many new fibers were found in the scarred area. These were placed in confusion for the most part and paralleled the

connective tissue strands (Fig. 4), ineffectually crossing from one side to another. They followed devious pathways to avoid connective tissue bundles, hemorrhages, and other obstacles and, if no other opening was found, recurved on themselves at a 180° angle (Fig. 5), like regenerating fibers in a peripheral nerve stump. Occasionally, however, thin bundles of nerve fibers could be traced across the whole scar from one stump to the other. Some of these new fibers clearly originated from spinal roots (Fig. 6), while others as surely came from the longitudinal columns in the spinal cord (Fig. 7). Thus, there was definite continuity in one case (RSP 11) of the severed left lateral column; and in another (RT 4) of the ventral and lateral columns.

With either muscle or nerve interposed between the cord ends, the growing nerve fibers were influenced. Muscle fibers placed transverse to the cord axis prevented longitudinal nerve growth; but when the orientation of the muscle fibers was parallel to the direction of the cord, axone growth across the gap was definitely improved (Fig. 8). The nerve implants served likewise as a growth bridge and new nerve fibers followed faithfully the path laid down by the degenerated ones, even when this curved back in a semicircle (Fig. 9).

The correlation between the anatomical and physiological pictures in individual animals was excellent. Absence of reflexes and an automatic bladder was always related to a degenerated caudal cord segment. The typical spinal animals, with strong reflexes but none of the further accomplishments, possessed healthy cord stumps separated by a scar with interlaced nerve fibers not continuous between them. The thirteen rats that showed physiological recovery had new fiber bundles connecting the cord stumps across the scar and, roughly, the amounts of restitution of structural connections and functional capacities were alike. Thus, the rat (RSP 11) that used its left hind-leg in righting itself had good continuity of the lateral column on that side; and one (RNC 3) with considerable "voluntary" leg movement and good responses to stimulation of the peduncles had many new fibers (running in a degenerated sciatic) connecting the cord stumps.

Two cases of incomplete cord section, already referred to, are also instructive; for the functional recovery far outdistanced the anatomical. Rat RSP 13, had voluntary leg movement on the day after operation, and proprioception and motor control returned during 15 days, when the animal could balance itself on its hind-legs and wipe its nose free of vaseline with both fore-paws in normal fashion. Histologically, the right ventral horn and lateral column were intact, but both posterior columns had been completely sectioned. The scarred area contained many new nerve fibers, some from a regenerating posterior root, and some clearly arising from columns of the spinal cord. The return of proprioception or other sensation from the legs and tail makes it certain that some of these new nerve fibers were functioning. Rat RT 15, had the entire dorsal half of the cord sectioned, down to the central canal. Nevertheless, after about four weeks, this animal had considerable proprioception, and by two months was walking almost normally.

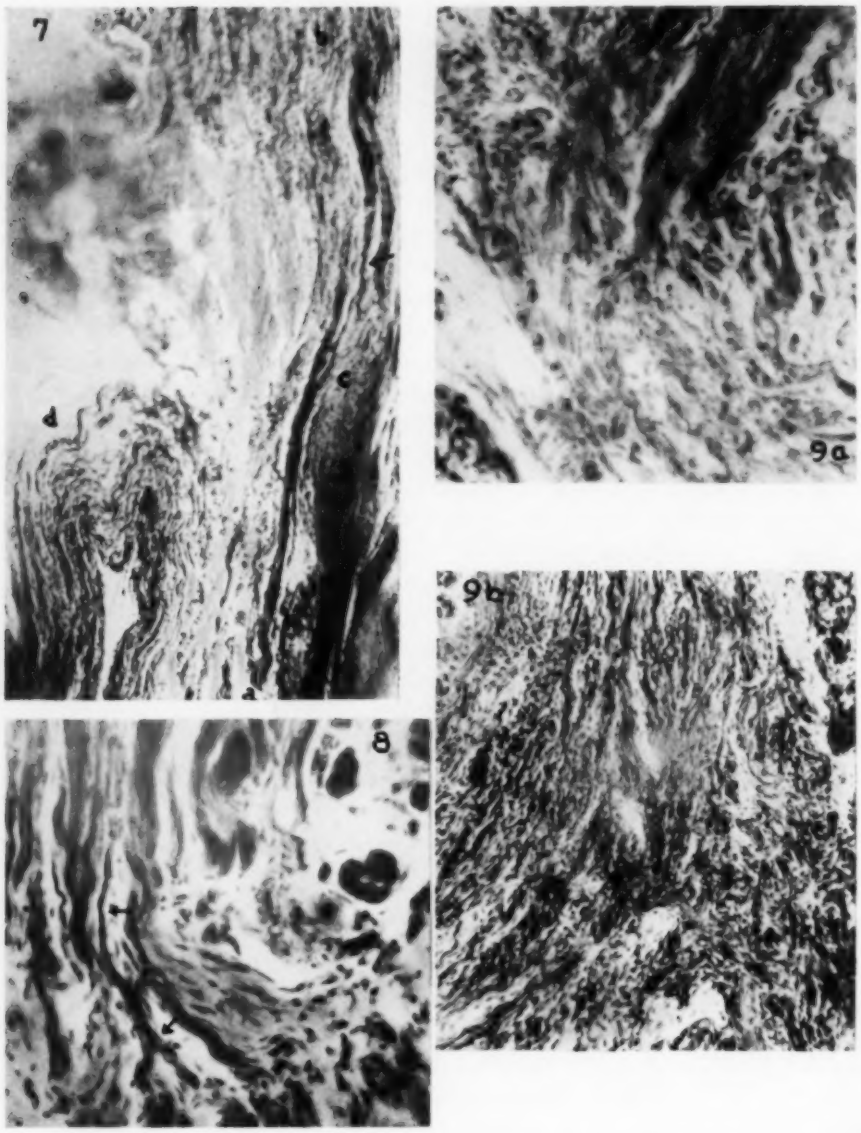

FIG. 7. Origin of new fibers (*a*) from lateral columns (*b*). Arrow points to bundle of new fibers splitting to avoid the large connective tissue mass (*c*). At (*d*) is a large group of fibers from the lower stump, recoiling at 180°. The large out-of-focus mass at the upper left is connective and degenerating embryonic transplanted tissues; ×80.

FIG. 8. Orientation of new nerve fibers along longitudinally arranged interposed muscle fibers. Arrow points to nerve bundle, ×240.

FIG. 9a. Regenerating cord fibers running into diagonally placed transplant of degenerated nerve. The dark material at the upper right is the transplant, in which may be seen fibers, which arise in the scar tissue below; ×240.

FIG. 9b. Regenerating fibers curving with curved nerve transplant. Continuation of Fig. 9a, with fibers originating below lower left; ×125.

Histologically, regenerating fibers connecting the cut ends were seen. Similar functional recovery correlated with appropriate anatomical regeneration occurred in the later series of partially sectioned rats.

Discussion

The disagreement between the histological results reported here and those of some of the other workers seem to depend primarily on the methods used. Hematoxylin and eosin, or toluidin blue and erythrosin, while invaluable for studying general structure and cellular alterations, are virtually useless in tracing newly formed nerve fibers. Since, in long-term experiments, normal-appearing cells (on Nissl and silver staining) may be present within 0.2 mm. of the scar, and since the return of function might be due entirely to new fiber paths between the two spinal cord stumps, a silver stain for nerve fibers seems imperative. Migliavacca (1932) has criticized the anatomical findings of Hooker and Nicholas (1930) for the absence of silver methods. Further, serial sections in the longitudinal plane are essential for tracing fibers between stumps. The inadequacy of cellular staining is clearly shown by comparing adjacent sections stained alternately with toluidin blue and erythrosin, and with silver, for only the latter brings out new fibers wandering into or across the scar (Fig. 3 and 4b).

Silver stains in general do color connective and collagenous tissue as well as nerve, but the method used gives clear contrasts and, as checked with known nerve fibers, the neural elements can be unequivocally identified. The uncertainty of block impregnation methods and the frequent necessity for discarding sections near the surface is well known. The silver-on-the-slide method of Bodian (1936, 1937) allows uniform and repeatable staining with silver, and selection of desired sections for cellular stains. (After formol fixation, even the iron-hematoxylin method of Mahon can be used.)

On the physiological side also, much of the earlier work has been methodologically unsatisfactory. Complete cord section affords the best test for regeneration since in partial lesions, as shown above and previously (Foerster, 1930; Gerard and Grinker, 1931), extensive vicarious functioning simulates anatomic repair. Yet "blind" cord section—through the dorsum of an embryo (Hooker and Nicholas, 1927, 1930; Gerard and Grinker, 1931; Migliavacca, 1930a, b) or between vertebrae (Cajal, 1928)—entails the danger of sparing part of the cord unless, to avoid this, excessive lesions are produced. Further, direct visual proof of the section is lacking in closed operations; and a trapped hemorrhage, regularly present, forms a barrier to fiber growth. Section of the exposed cord after laminectomy has been little used and then in conjunction with inadequate staining (*e.g.*, Yamada, 1907). Placing gauze between the cut ends (Ssamarin, 1926), while efficiently preventing hemorrhagic barriers, undoubtedly causes walling off of the ends and organization of the connective tissue in directions unfavorable for regeneration. Mechanical section with a clean sharp knife stroke causes less ultimate tissue damage than contusion, coagulation, or freezing. The elec-

trocautery produces large cysts (Marburg, 1936) and degeneration of multipolar cord cells, even spinal ganglion cells, for several segments to either side of the lesion (Hooker and Nicholas, 1930); while following a sharp cut and full hemostasis normal cells remain within less than 0.5 mm. from the scar (Fig. 2).

In the present series, after traction on the tail was introduced, there was no single case of failure to section the cord completely, as shown by three independent kinds of evidence: the cut ends were seen to separate by 2 to 3 mm. after the knife was passed; no sign of ascending or descending transmission across the level of the lesion was present within 3 to 4 weeks of operation; serial sections showed a scar extending completely across the cord.

The true spinal rat shows considerable reflex activity which reaches an asymptote by one month after the lesion is placed. Galamini (1931) sectioned the cord above the lumbar enlargement and reported loss of bladder and rectal sphincter tone for a week, a bilaterally unequal loss of leg muscle activity and, between 2 or 3 weeks and the same number of months, a progressive return of coordinated movement. Since the author, himself, attributes differences in tone in the two legs to "asimmetria della lesione prodotto con l'intervento chirurgico" and since no details of operation or subsequent testing are given it seems likely that these were cases of incomplete section—as in some of our preliminary animals. Hooker and Nicholas (1930, p. 447) reported that "spinal animals possess the capacity to perform coordinated movements, and the definite transmission of excitation and response in both directions past the region of spinal section" and attributed this pasage to collateral paths via proprioceptive arcs that bridged the lesion. We have not seen such transmission or coordination in our spinal animals; and the collateral path hypothesis is ruled out by the peduncle stimulation experiments, for vigorous contractions anterior to the section never led to movements posterior to it in true spinal animals.

In sharp contrast to these permanently spinal animals is the group of 13 which, after a typical spinal period, later acquired definite transmission past the level of section. This is evidenced by the return of coordinated movements, sensation and, most directly, by hind-quarter responses to electrical stimulation of the upper brain stem. Unfortunately, the ascent of impulses set up by direct stimulation of the caudal cord was not tested; and the evidence of conscious sensation is only partial. (Failure to vocalize in response to moderately painful stimuli is common in trained or habituated animals). The polysynaptic sensory paths may, of course, have recovered less than the simpler motor ones.

In every one of these animals showing functional recovery, there were newly formed nerve fibers connecting the cord stumps across the scar. These were present in considerable number, forming many small bundles, and could be traced between some cord tract above the lesion and one below. The finer correlation of particular kinds of functional return wlth good fiber repair of the appropriate anatomical tracts is further evidence in the same

direction. The occasional presence of bridging nerve bundles in animals that failed to show functional recovery (3 cases) is not, of course, in conflict with the above. These fibers were always sparse and sometimes originated from roots or, presumably, made unfortunate connections. The conclusion seems inescapable that nerve fibers can grow from spinal elements across a region of complete discontinuity and can then conduct nerve impulses which restore transmission across the lesion and some degree of normal function.

Some further consideration of the source of the regenerated fibers is required because the remote possibility exists of a functional bridging of ununited stumps by fibers growing from sectioned roots distal (or proximal) to the cut and reaching the proximal (or distal) cord stump. In the total cord sections, roots were cut and did indeed contribute new fibers, though the ventral roots participated but little. Dorsal and some ventral fibers usually followed the scar transversely but bundles of them could, not uncommonly, be traced longitudinally well into a cord stump, where they ended in growth cones or were lost among other fibers in the white columns. (Cajal, 1929, p. 533, had traced root fibers to the stump after 5 days but did not follow them further). Such root fibers, besides offering a possible conducting path for impulses, also might serve as trail breakers for other cord fibers later growing by their sides.

Whatever contribution regenerated root fibers may make, however, they are certainly in no way vital to cord regeneration. Even in total sections it was often possible to follow bundles of axones from a cord tract in one stump across the scar into grey or white of the other stump. This was especially clear in cases with nerve implants in which root fibers could be shown not to come into question. Further, ventral roots were rarely cut and, in the special series with partial sections, no roots were injured; yet the same new fibers completely crossing the scar were readily seen. (Some lateral herniation in these cases makes it especially easy to trace the regenerated fibers). Groups of 2 to 5 counted up to hundreds of fibers in a single partial transverse section; and, since relatively more new growth follows large lesions than small ones (Cajal, p. 510), a really considerable number must cross a complete section.

The practical success of cord regeneration (and presumably any central regeneration) is determined by the fibers growing across the lesion. Many growing axones penetrate into the scar tissue (see also Ssamarin, 1926; Cajal 1928) but more rarely do they cross it (Gerard and Grinker, 1931). To encourage this successful bridging, we tried implants into the cut of embryonic nervous system, of adult muscle, and of degenerating nerve, which might liberate "neurotropic" substances or supply a material scaffolding. The embryonic nerve cells rarely survived and then mainly as acinar clumps. Perhaps still younger embryo tissue might adapt itself and grow its own fibers to knit in with the injured cord; in two of our cases, however, definite neurone development occurred. It is interesting that Willis (1936) was able to culture almost any embryonic tissue except nervous tissue as intracerebral implants.

Muscle and nerve definitely serve as scaffolding for growing nerve fibers, which follow the direction of the implanted elements. All our best cases of regeneration had such an implant, mainly nerve which retained a favorable orientation. It should prove possible, with greater care in placing and fixing a nerve implant, to secure more regular and complete spinal regeneration.

Degenerated peripheral nerve has long been used as a bridge in efforts to encourage neural regeneration. Marinesco and Dustin (see Cajal, 1928) employed it in peripheral nerve gaps; Loez and Arcaute (1913) in sectioned optic nerves; and Tello (1911, 1924) in cut cerebral white matter. In each case, new axones grew into the degenerated paths. Celloidin blocks (Oiye, 1928) and elder pith (Tello, 1924) have also been used in studies of central regeneration, with indications of axone growth. The present results seem to hold more promise of clinical application than did the early ones.

Cajal (1928) attributed failure of central regeneration to lack of pathways and of neurotropic substances; others (Spatz, 1930; Yamada, 1907) have blamed the absence of Schwann cells. Weiss (1934) has urged, especially for the outgrowth of new fibers in embryos, that fibers follow the structural paths of least resistance. Our results support this view. Individual fibers avoid obstacles and follow surfaces (a positive thigmotropism)—including blood vessels (Shirai, 1935),—and even recurve with them. That the Schwann cells are not essential to fiber regeneration is attested by the outgrowth of peripheral axones when Bands of Bugner are missing (see Weiss, 1934, and Rossi and Gastaldi, 1935) or in advance of the Schwann cells into scar tissue, in tissue culture (Harrison, 1908), and in initial growth (Harrison, 1924; Speidel, 1932). On the other hand, Schwann cells certainly aid regeneration in peripheral nerve; and in a few striking cases glial cells were seen to have formed in "bands" along regenerated central axones in a fashion analogous to that of Schwann cells in the periphery (Fig. 10).

The common failure of central regeneration is primarily due to improper pathways for the regrowing nerve fibers. This is a consequence of the early and rapid growth of glia and connective tissue to form an impenetrable mass of haphazardly arranged fibers and cells before the nerve regeneration gets well under way. This is further evidenced by experiments (Sugar and Gerard, unpublished) in which the central ends of cut peripheral nerves were led into the cerebral cortex. New fibers grow out into the cerebral tissue but are eventually blocked by a tremendous accumulation of glia and macrophages which results from the injury and the presence of a foreign body.

Regeneration depends primarily, of course, on the presence of viable neurones not too far from the lesion which must be bridged by new axones. Insufficient blood supply to the cut ends of the cord is often an additional factor hindering regeneration. The cranial stump almost always receives enough blood from the vertebrals via the spinal arteries, but the caudal stump usually depends on aortic branches for its supply. If these branches are cut, as in sections below T13, the caudal stump suffers degeneration and of course does not show repair. The choice of lumbar and sacral levels for

placing lesions (*e.g.*, most of Cajal's) is thus unfortunate and handicaps successful regeneration.

In higher animals the meagre collateral circulation of the cord militates against regeneration. Thus, in the cat (Tureen, 1936), occlusion of the ab-

FIG. 10. Drawing of glial cell "band" accompanying regenerating central axones crossing hemisection. a. Bundle of new fibers arising in upper segment of cord with fibers of lateral column (b); c. glial cell nuclei.

dominal aorta causes degeneration of the spinal cord below the occluded level though the anterior spinal artery is intact. Conversely, paralysis from occlusion of the anterior spinal artery is well known in man (Zeitlin and Lichtenstein, 1936). In the rat, however, section of the anterior spinal artery

per se (cord lifted out of the way) is not followed by permanent damage to the cord, and the same is reported for the goat (Hunter and Royle, 1924). Poor blood supply probably accounts for the rather frequent failure of spinal humans (Riddoch, 1917) and monkeys (Sherrington, 1900) to develop proper spinal reflexes. Indeed, as blood supply is essential for normal neural function (Gerard, 1938), so it must influence regenerating vigor; and quantitative variations in vascularity may help control the degree of regeneration at different loci in the nervous system and in animals of different age and species.

As a spinal mammal, the rat seems to occupy a position on the phylogenetic scale slightly above the rabbit (Hinsey and Cutting, 1933) and definitely above the opossum (Hinsey and Cutting, 1936), but distinctly below the dog and cat (Sherrington, 1900) and the monkey (Hinsey and Markee, 1938). Such a rating is based on the time required for regaining spinal reflexes and bladder automaticity, and on the degree of spinal shock. Shock is almost transitory in the opossum, but enduring in the carnivores and higher animals, thus paralleling the degree of cephalization (Hinsey and Cutting, 1936; Fulton, 1938). It also varies inversely with blood supply; and work is in progress on a possible anemic factor in its production.

Summary

1. Spinal rats (cord section between T5 and T13, with tension on the tail causing the cut to gape 2–3 mm. and insuring a complete lesion) will live indefinitely (8 mos.) with moderate care.

2. Edema and gangrene of the hind-legs (as well as hydronephrosis and hydroureters) are the main cause of early death and depend directly on urine retention. Manual expression of urine 3 times a day during the period of returning bladder control, 7 to 10 days, eliminates this difficulty.

3. Flexion reflexes are present immediately after section, crossed responses appear after 3 days, scratch reflexes after 11 days. Complex and fractional responses of hing-legs and tail develop, but with no sign of coordination with the front quarters.

4. By one month, the typical spinal animal has reached a steady level of behavior and fails various tests of sensation and voluntary motor control. At the time of sacrifice electrical stimulation of the cerebral peduncle does not evoke motor responses below the level of section. Cell stains show normal neurones close to a complete transverse scar and silver methods reveal new axones entering and becoming tangled in the connective tissue of the lesion.

5. In some cases (13 in all), after four weeks of typical spinal performance, further sensory and motor recovery occurred, including voluntary climbing, walking and hopping movements, and placing and seeking based on good proprioception. At sacrifice, stimulation of the brain stem produced hind-leg movements. Silver stains showed a complete scar bridged by bundles of new axones passing continuously between cord tracts on either side of the lesion. The fibers entering and crossing the scar ordinarily arise

from both the cord tracts and spinal roots, but when the latter are excluded physiological recovery still occurs.

6. The most marked recovery was in rats with properly oriented nerve or muscle implants in the cord gap. Recovery was poor with embryonic brain implants; which degenerated, formed acinar cell stumps, or developed into small pyramids.

7. Spinal neurones with adequate blood supply start to regenerate cut processes. These fibres grow along structural pathways like peripheral nerves and using bands of glial nuclei when possible, but are mainly blocked by glia and scar tissue running transversely across the cord. When they successfully cross a scar, restoring anatomical continuity, nervous transmission across the lesion and coordinated function also return.

8. True anatomical and physiological regeneration can occur in the rat spinal cord. This is aided by an implant of degenerating sciatic nerve. Such implants may find clinical application.

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PALEOCEREBELLAR INHIBITION OF VASOMOTOR AND RESPIRATORY CAROTID SINUS REFLEXES

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INTRODUCTION

THE PROBLEM of cerebellar function is still controversial. It is agreed, however, that the cerebellum acts in relation to volitional movement and therefore may be considered a center of *somatic* nervous activity. The possibility of an action on the *autonomic* functions has often been considered. A complete review of the literature on the subject was given in a previous paper (Moruzzi, 1938). One need only recall here that this action has been conceived of in two different ways: (i) as an influence of the cerebellum on voluntary muscles through the intermediation of the autonomic nervous system (Camis, 1913; Krestovnikoff, 1926); or (ii), as a direct cerebellar action on visceral organs. The object of the present work is to investigate the latter possibility.

This second relation was particularly stressed before the work of Luciani. Afterwards the theory was almost abandoned, but has been taken up again recently by different authors and notably by the Orbeli School in Russia. Experiments so far carried out on this subject have not appeared convincing perhaps owing to the fact that the results were often contradictory and open to technical objections (spread of physical stimuli in the excitation experiments, secondary lesions of neighboring centers after total or localized cerebellar extirpations, indirect effects caused in a reflex or purely mechanical manner by changes in the activity of the striated muscles). In any case these experiments have played little part in the formulation of recent doctrines on cerebellar physiology (Miller, 1926; Van Rijnberk, 1931; Bremer, 1935). Conclusions drawn from them have been formally discarded by other authors (Dusser de Barenne, 1937; Karplus, 1937). Nevertheless Fulton in a recent review (1936) insists on the fact that in the premotor area of the cerebral cortex the somatic and vegetative functions have a focus of common integration; in view of the close relation between the cerebral cortex and the cerebellum, it would not be surprising if "further investigation would lead us to a conception of ataxia and asynergia in autonomic regulation similar to that now held for cerebellar disturbances in the somatic sphere."

We have recently (1937, 1938) examined the function of the paleocerebellum in relation to a fundamental activity of vegetative life, the nervous regulation of vasomotor tonus. These experiments, which were carried out on precollicular cats, were concerned exclusively with the excitable area of

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the paleocerebellum. This area coincides, as Bremer (1922) has shown, with that part of the paleocerebellar cortex which forms the site of termination of Flechsig's and Gower's tracts and thereby receives the name of "spinal story" (Ingvar) or "Pars somatica" (Ariens Kappers) of the cerebellum. In our experiments, therefore, we decided to determine whether a weak faradic stimulation of the excitable zone of the paleocerebellar cortex, which has long been known to inhibit extensor posture of extensor muscles (see Bremer, 1922 and 1935), is capable of inhibiting the vasomotor action.

The results were distinctly positive. They have shown that: (i) weak faradic stimulation of the paleocerebellum (vermian part of the anterior lobe) strongly inhibits the vasopressor reflexes caused by electrical stimulation of a sensory nerve (superior laryngeal, sciatic); (ii) similar paleocerebellar faradization inhibits vasodilator reflexes evoked by electrical stimulation of the central end of the vago-depressor nerve (after double vagotomy; (iii) spontaneous vasomotor waves are equally inhibited; (iv) paleocerebellar stimulation has a depressor action on blood pressure, when the latter is spontaneously raised by a central mechanism (strong bulbar vasomotor tonus), the action failing if blood pressure is low, or raised artificially by a peripheral mechanism (injection of ephedrine); (v) inhibitory action is still present in curarized animals; moreover the depressor action mentioned above in (iv) is sometimes present with stimulation so weak that it is without action on extensor posture paleocerebellar inhibition is thus independent of the classic inhibiting action on postural extensor tonus; (vi) superficial cocainization of the excitable cortex suppresses the inhibitory action; the latter thus has a corticocerebellar origin.

We conclude from our experiments that there is a direct inhibitory action of the paleocerebellar cortex on the central vasomotor center and that, in consequence, it must be admitted that the cerebellar function affects, not only the somatic nervous activity, but also the autonomic nervous system. It is a well known fact that in the brain stem are the centers of a highly developed reflex vegetative activity, which constantly control the respiration and circulation. In this respect the carotid sinus reflexes play an essential part. It was therefore of interest to examine whether these reflex activities are subject to the control of the paleocerebellum. For the literature on the carotid sinus reflexes we refer to the monographs of Heymans, Bouckaert and Dautrebaude (1933) and of Heymans and Cordier (1935).

An action of the cerebellum on the carotid sinus reflexes has not, to our knowledge, been considered until now. It has been stated recently, however, by Mansfeld and Tyukodi (1936) that the cerebellum, and not the brain stem, would be the center of carotid sinus chemical reflexes acting on respiration. Mansfeld and Tyukodi base their statement on experiments involving decerebration and extirpation of the cerebellum; they observed in fact that after the latter operation the carotid sinus chemical reflexes on the respiration disappeared.

The experiments of Mansfeld and Tyukodi were repeated by Stella

(1937), who did not confirm them. Mansfeld and Hamori (1938) on the other hand have recently insisted on the absence of these chemical reflexes after decerebration and decerebellation. But in a demonstration at the general meeting of the physiological society (1939), Stella has again clearly shown the presence of strong respiratory reflexes from the carotid sinus chemo-receptors in a decerebrate and decerebellate, bulbo-protuberantial dog. The positive results obtained by Stella are decisive evidence against the hypothesis of Mansfeld and his collaborators. The negative results of the latter authors can easily be explained if one considers the close relations (topographical and circulatory) between cerebellum and brain stem, the acute and severe character of the operation, the low level of the decerebration.

It may thus be concluded that vegetative, as well as postural (Magnus, 1914), reflexes are still present in the decerebellate animal; the anatomical localization of all these reflex arcs is chiefly bulbo-pontine. The question then arises whether there is an action of the cerebellum on the underlying bulbo-pontine reflexes. With regard to the postural tonus, it is generally admitted that this is so. Our experiments suggest that, at least so far as the paleocerebellum is concerned, the same conclusions are true in regard to two fundamental vegetative activities, *i.e.*, the vasomotor and respiratory carotid-sinus reflexes.

METHODS

The investigations were carried out on precollicular cats. In order to reduce to a minimum the time of occlusion of the common carotid arteries during the decerebration, we used the following technique, suggested to us by Professor Bremer. A thalamic preparation was made under ether anaesthesia, followed immediately by a precollicular decerebration, without removal of the diencephalon. In this way it was possible to prevent injury to the large arteries at the base of the skull, and to reduce considerably the danger of carotid hemorrhage.

Electrical stimulation of the vermian part of the anterior lobe of the cerebellum was bipolar and faradic (induction coil; about 50 shocks per second). The intensity of the stimulation was, in general, that which provoked the classic inhibition of decerebrate rigidity (*i.e.*, immediately above the threshold level for the tongue). Blood pressure was recorded from the femoral artery in the ordinary manner and respiration by means of a Marey capsule joined to the trachea.

Experimental Results

Inhibition of vasomotor carotid-sinus reflexes by faradic stimulation of the paleocerebellar cortex

Occlusion of one or both of the common carotid arteries is followed by a marked increase in blood pressure and respiration (Pagano-Hering's reflexes). The blood pressure increase is chiefly due to a release of the bulbar vasoconstrictor center from the inhibitory impulses arising in the carotid sinus pressoreceptors. The hyperpnea, at least in the decerebrate preparation, is also related to a reflex excitation of the respiratory center from the carotid sinus chemoreceptors (Stella, 1936). In both cases the background of increased central activity is a very favorable condition for demonstrating an inhibitory action of the paleocerebellum on the bulbo-pontine vegetative

centers; in the same way that the background of increased activity, which is the characteristic feature of the bulbo-pontine tonic centres in the decerebrate preparation, is a very good condition for demonstrating the inhibitory action of the paleocerebellum on the postural tonus.

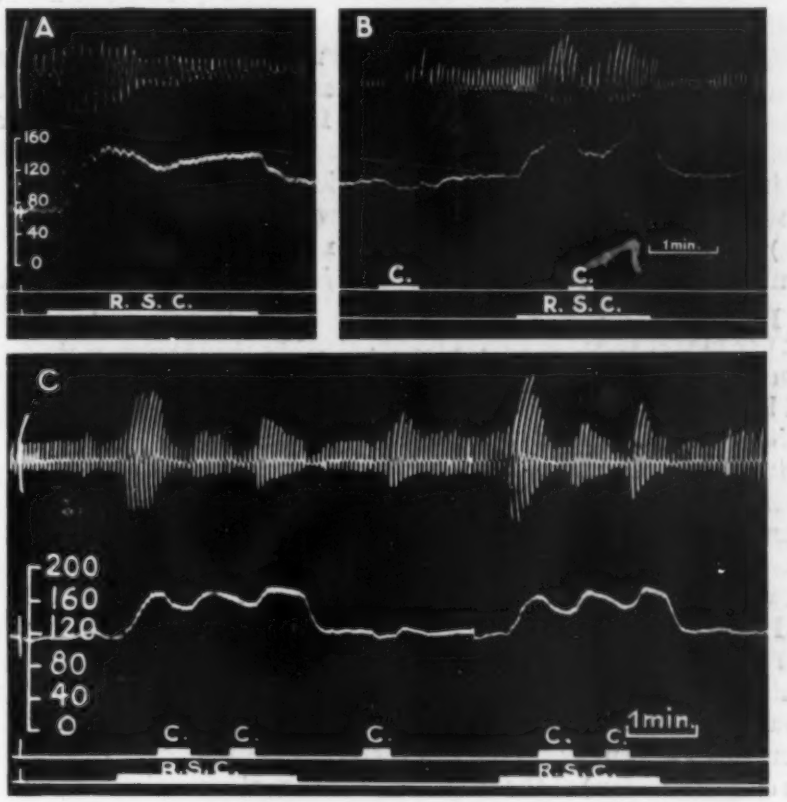

FIG. 1. Action of paleocerebellar stimulation on vasomotor and respiratory carotid-sinus reflexes (provoked by the occlusion of the common carotid arteries). In each record from above downwards: respiration; arterial blood pressure; c, signal of faradization of anterior lobe; R.S.C., signal indicating bilateral occlusion of common carotid.

A. Carotid-sinus reflex of control.

B. Effects of a paleocerebellar stimulation on normal blood pressure and respiration (left); effects of the same stimulus on carotid-sinus reflex (right) (R.S.C.).

C. Another preparation, same type of experiment. Note in B and C the small paleocerebellar depressor effects when blood pressure is not raised by carotid occlusion. The inhibition of postural extensor contraction was the same in each case.

With regard to the action of the paleocerebellum on the vasomotor tonus, we found that weak faradic stimulation of the vermis (lobus anterior) is followed by a marked decrease in blood pressure which had been raised by the occlusion of the common carotid arteries (Fig. 1). The depressor effect may be so strong as to abolish the Pagano-Hering's effect. The inhibitory action is reversible: cessation of the cerebellar stimulation is followed by an

increase in the blood pressure (Fig. 1). It is thus possible, in the course of a few seconds and during the same carotid sinus reflex, to obtain repeatedly the paleocerebellar depressor actions (Fig. 1). We may conclude from these observations that faradic stimulation of the paleocerebellum can counteract, at least in so far as the blood pressure is concerned, the carotid sinus (Pagano-Hering) reflexes.

This antagonism between carotid sinus reflexes and paleocerebellar stimulation is not satisfactory evidence of a central inhibitory effect of the latter. Decrease in blood pressure may result from many different mechanisms. Two observations however strongly support the hypothesis of a paleocerebellar inhibition of the bulbar vasoconstrictor tonus: namely, faradic stimulation of the paleocerebellum has no depressor effect, or only a very feeble one (a) when the blood pressure is low or normal, *i.e.*, not raised by the occlusion of the common carotid arteries (Fig. 1B, 4, 5), and (b) when a low blood pressure is increased by injections of drugs (ephedrine) acting chiefly on the peripheral vasoconstrictor tonus. We may thus conclude that, in order to produce a paleocerebellar depressor effect, the blood pressure must be raised by a central mechanism, *i.e.*, it is necessary to produce a background of excitation in the bulbar vasoconstrictor center. These experimental facts are actually inconsistent with the hypothesis of a pure peripheral antagonism between paleocerebellar stimulation and carotid sinus reflexes, the former decreasing and the latter increasing blood pressure.

The inhibitory action of the carotid sinus circulatory reflexes, produced by faradic stimulation of the paleocerebellar cortex, is not necessarily correlated with the well known inhibition of the decerebrate rigidity produced by the same stimulation. As a matter of fact the vegetative action is still present in curarized animals (Fig. 2), after complete abolition of the peripheral paleocerebellar somatic effects. The vegetative inhibition is thus an independent phenomenon and not merely a consequence (by mechanical means or reflex action) of the inhibition of the postural extensor tonus.

Double cervical vagotomy does not abolish or decrease the inhibition; nor is the paleocerebellar depressor effect characterized (when the vagal innervation is still present) by any important changes in the heart beat frequency. The inhibition thus chiefly affects the vasomotor centers.

Local cocainization (cocaine chlorhydrate 2–5 per cent) abolishes the inhibitory effect on vasomotor tonus, as it does the inhibition of postural tonus. The paleocerebellar faradization effects are thus primarily due to the activity of the faradised cortex. Strengthening the intensity of the stimulation is however followed by reappearance of the inhibitory effects previously abolished by local cocainization; the phenomenon is probably due to spread of physical stimuli to the deep cortical convolutions and to the cerebellar nuclei. It must be remembered here that so far as postural tonus is concerned the activity of the cerebellar nuclei seems to be very similar to that of the cerebellar cortex (Miller and Laughton, 1928; Magoun, Hare and Ranson, 1935). We may conclude from these experiments that the paleocerebellar cor-

tex clearly inhibits the central vasomotor tonus. It is now of interest to examine the main similarities and differences between the well known somatic action of the paleocerebellum on postural tonus and the vegetative effects on vasomotor centers.

There is considerable parallelism between the two paleocerebellar inhibitions: (i) the effects are constantly produced by weak faradizations of almost the same intensity and duration and applied to the same areas of the cerebellar cortex; (ii) in both cases the inhibitions are slow to appear, they

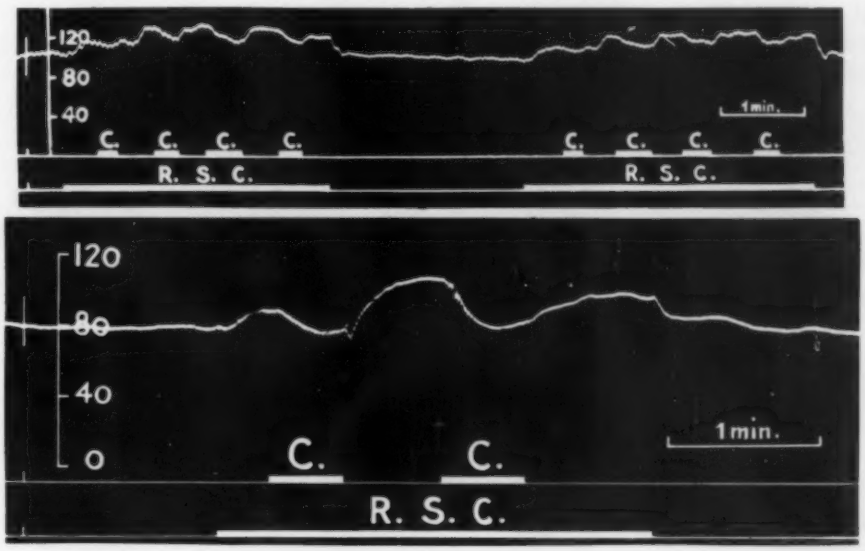

FIG. 2 (*Above*). Action of paleocerebellar stimulations on vasomotor carotid-sinus reflexes in a curarized animal. Same type of experiment as in Fig. 1, but during a curare paralysis (intravenous injection; 1 mg/kg). Owing to the initial small carotid-sinus reflex, the first paleocerebellar stimulation of every series has no depressor effect; paleocerebellar rebound increases blood pressure.

FIG. 3 (*Below*). Action of paleocerebellar stimulations on a feeble vasomotor carotid-sinus reflex. Same type of experiment as that shown in Fig. 1. Small depressor effect at the first paleocerebellar stimulation. The rebound increases the blood pressure level; a new paleocerebellar stimulation is followed then by a more marked inhibitory action.

persist during the whole time of stimulation and are followed by marked rebounds; (iii) if the background of activity to be inhibited is small or absent, there is little or no apparent paleocerebellar effect during the stimulation, but the rebound is extremely strong and persistent; a new stimulation strongly inhibits the rebound and is followed by another (sometimes still stronger) rebound (see Bremer, 1922; Bremer and Ley, 1927, Moruzzi, 1936 for the postural tonus; see Fig. 3 for vasomotor tonus).

The main difference between the somatic and the vegetative activities of the paleocerebellar cortex is that the intensity of the paleocerebellar influence seems to be much greater for postural contraction of skeletal muscle

than for vasomotor tonus. As a matter of fact it is always possible to produce a complete paleocerebellar abolition of the postural extensor tonus; so far as the somatic control is concerned, the faradic stimulation of the vermis ceases to inhibit only when there is no more background of central activity to inhibit. Quite different is the paleocerebellar behavior towards vasomotor tonus. There is no depressor action (or only a feeble one) on the normal blood pressure. Faradic stimulation of the excitable vermian cortex may however (as we have seen) abolish the pressor effect of the Pagano-Hering reflex; but in this case too the blood pressure does not fall, under the action of the paleocerebellar stimulation, definitely below its normal level. In other words paleocerebellar stimulation ceases to inhibit when there is still considerable central (spinal and bulbar) vasoconstrictor tonus.

Inhibition of respiration and of respiratory carotid sinus reflexes by faradic stimulation of paleocerebellar cortex

Faradic stimulation of the paleocerebellar cortex has a constant and definite inhibitory effect on the respiration of the decerebrate cat (Fig. 1b). Both the frequency and the amplitude of the respiratory movements are decreased; the inhibition is sometimes followed, at the end of the stimulation, by a powerful rebound which chiefly affects the depth of the inspiration.

The inhibitory effects are much more evident if the respiration has been increased by a carotid sinus reflex. We have investigated the action of a paleocerebellar stimulation on the hyperpnea produced by the occlusion of the common carotid arteries or by intracarotid injections of KCN. Both these hyperpneas are reflex in nature (Pagano, 1900). The first is simply the respiratory aspect of the Pagano-Hering's reflex; the second is due to the excitation by KCN of the carotid body chemoreceptors (Heymans, Bouckaert and Dautrebande, 1931; Heymans and Bouckaert, 1932; Samaan and Stella (1935)).

The general features of the inhibition are the same as those already described for normal respiration. During the persistent hyperpneas produced by occlusion of the common carotid arteries (Fig. 1) or by intracarotid injections of large doses (0.3–0.7 mg) of potassium cyanide (Fig. 4B, 5), it is possible to obtain the inhibitory effects repeatedly and constantly. The respiratory reflexes of short duration produced by intracarotid injections of small doses (0.1 mg) of KCN, are on the other hand much less strong when the injection is made during a faradic stimulation of the paleocerebellum (Fig. 4A).

The paleocerebellar inhibition of respiration is due, like the inhibition of postural or vasomotor tonus, to the activity of the cerebellar cortex which is faradized; in fact local cocainization abolishes the inhibition.

On the other hand the effects on respiration are quite independent of the well known inhibition of decerebrate rigidity. As a matter of fact the former is still present when the latter is completely absent owing to the abolition of postural tonus produced by small doses of curare ("atonie curarique" of

Bremer, Titeca and van der Meiren, 1927). These experiments, like those on vasomotor effects in curarized animals, do not reject the possibility that in normal animals the powerful inhibitions and rebounds in the somatic activity may have some secondary effects on blood pressure or on respiration.* It

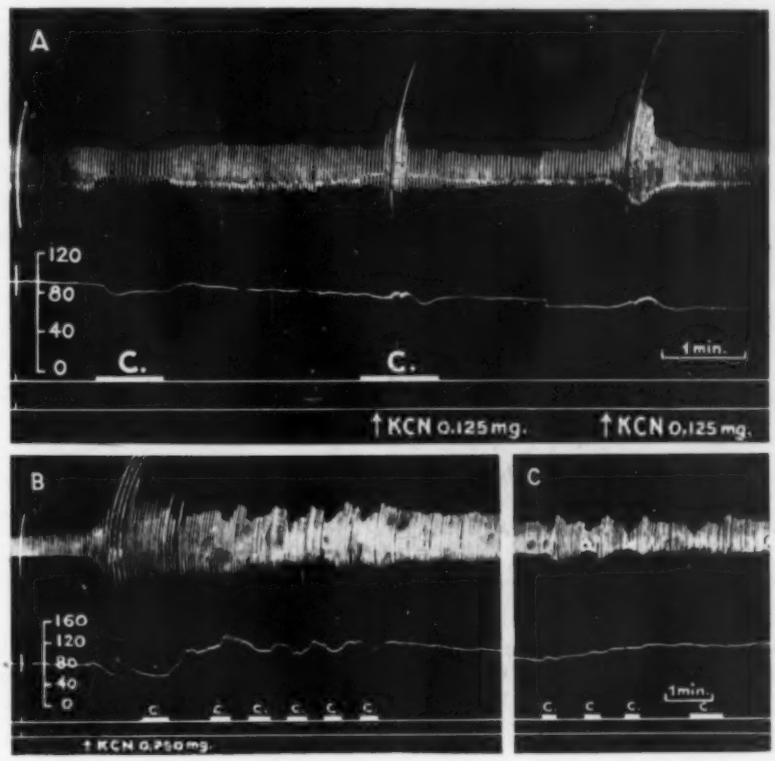

FIG. 4. Action of paleocerebellar stimulations on chemical respiratory carotid-sinus reflexes. Precollicular cat. Reflex activity provoked by intracarotid injections of potassium cyanide (arrow).

A. From left to right: action of a paleocerebellar stimulation (C) on normal respiration; inhibitory action of the same stimulation on a chemical respiratory reflex; respiratory reflex of control.

B and C (another preparation). Action of two paleocerebellar stimulations on a long respiratory reflex provoked by intracarotid injection of a large dose of potassium cyanide. The inhibition affects chiefly the amplitude of the respiratory movements.

must, however, be emphasized that these secondary actions, if present, are not the essential features of the inhibitory effects which we have observed. On the other hand the inhibition of respiration is independent of the inhibition of vasomotor tonus. It is, in effect, possible to have the former when the

* Clear examples of the relations between respiration and postural tonus are reported in Beritoff (1916), Olefirenko (1937) for the decerebrate preparation; in Barcroft and Barron (1937–38) for the foetal animal.

latter is absent owing to the low level of the bulbar vasomotor tonus (Fig. 1B, 4A and C, 5); and the reverse is sometimes true.

We may thus conclude that paleocerebellar stimulation is followed by (at least) three independent inhibitory effects; the well known inhibition of postural extensor tonus and the inhibitions of vasomotor tonus and respiration. The three effects are clearly due to the impulses arising in the paleocerebellar cortex and acting on bulbopontine centers of the postural and vasomotor tonus and of the resporation. It is, however, necessary to emphasize that the expression "independent inhibitory effects" is nothing but a pure translation in words of the experimental fact that it is possible to have one effect in the absence of the others. It does not mean that in paleocerebellar cortex some units (Purkinje cells) are specialized in the control of somatic functions, others in the control of the vegetative ones; nor that there are no central interrelations between all these activities. The contrary is probably true. It is only necessary to recall here (a) that all the inhibited centers have a bulbopontine localization and (b) that the existence of close functional (purely central) interrelations between these centres is highly probable (see Adrian, Bronk and Phillips, 1932, for the relationships between vasomotor and respiratory centers; Barcroft and Barron, 1937–38, for relations between postural tonus and respiration).

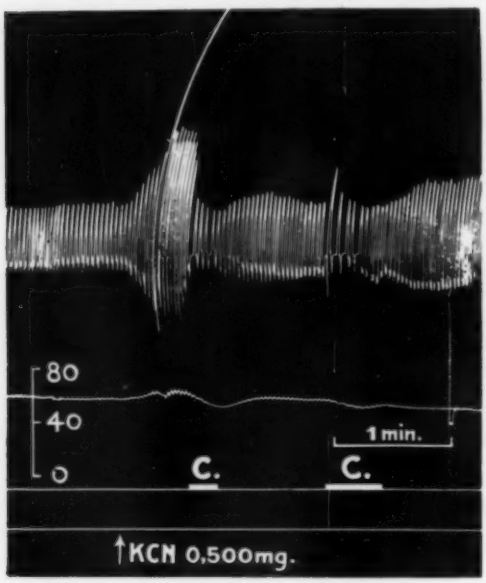

FIG. 5. Action of paleocerebellar stimulation on chemical respiratory carotid-sinus reflexes (produced by intracarotid injection of potassium cyanide). Signals as in Fig. 4. Paleocerebellar inhibition affects chiefly the frequency of respiration; blood pressure is low and therefore almost unaffected by the paleocerebellar stimulation.

DISCUSSION

The main result of these experiments is the demonstration of a cerebellar action on two fundamental functions of the vegetative life, namely circulation and respiration. Consequently the classic doctrine, according to which the cerebellum is purely a somatic center, is apparently too narrow. The vegetative function of the cerebellum must thus be taken into serious consideration. It should be emphasized, however, that the present experiments only concern the function of the "spinal story" of the paleocerebellum in the decerebrate preparation, *i.e.*, the activity of a comparatively small

part of cerebellar cortex in the simplest possible condition. Whether the present results are to be extended beyond these limits (neocerebellar relations between cerebellum and higher vegetative centers), is still an open question, which will possibly be settled by further investigation; it will not be dealt with here.

We know at present four different types of nervous activity which may be inhibited by a paleocerebellar stimulation. Of these two are somatic, two vegetative in nature. They are: (i) somatic postural contraction, *i.e.*, the inhibition of the decerebrate rigidity which is best known and still the most striking effect of paleocerebellar stimulation (see Bremer, 1935); (ii) effects on spinal reflexes (Bremer, 1922; Denny-Brown, Eccles and Liddell, 1929); this inhibition is still present after elective abolition by curare of the tonic component of the spinal reflexes (Moruzzi, 1935). This indicates that the phasic (kinetic) reflex activity is directly inhibited by the paleocerebellum; (iii) vasomotor tonus and vasomotor reflexes, (iv) respiration and respiratory reflexes. Of these functions three at least (i, ii and iii above) have a bulbopontine localization; thus the control of bulbopontine functions* seems to be the chief aim of the paleocerebellar activity in the decerebrate preparation.

A regulatory control of this kind can only be efficient if it is reflex in nature. The remarkable coincidence between the inhibitory area and the zone of cortical termination of the spino-cerebellar tracts (Bremer, 1922) strongly supports this view. The paleocerebellum may accordingly (and roughly) be described as a system of reflex arcs "in derivation" with the underlying bulbopontine (somatic and vegetative) reflexes. The problem we have to deal with is finally that of the relationships between the bulbopontine and the superimposed paleocerebellar reflexes.

It is of course impossible to give an adequate explanation of the mechanism of these correlations. But two points at least should be emphasized. First, the inhibitory function of the paleocerebellum is not necessarily continuous or "tonic" for all the bulbopontine activities. So far as the regulation of blood pressure is concerned it is quite possible, for example, that the highly developed bulbopontine mechanisms (namely carotid sinus and cardioaortic reflexes) perform the largest part of the work. On the other hand *inhibition* is not the only possible function of the paleocerebellum. The observations of augmentatory paleocerebellar actions on extensor muscles (Denny-Brown, Eccles and Liddell, 1929), chiefly when their postural tonus is absent (Moruzzi, 1936), as well as the paleocerebellar inhibition of vasodilator reflexes (Moruzzi, 1938), might possibly indicate that the aims of paleocerebellar activity should be described as a control more than a simple unidirectional inhibition of somatic and autonomic bulbopontine reflexes.

* That does not imply that all the bulbopontine reflexes are under a paleocerebellar control. Faradic stimulation of the anterior lobe does not inhibit the trismus (Bremer, 1935) or the deglutition reflex (personal observations) of the decerebrate preparation.

It is consequently probable that, in the last instance, the bulbopontine centers impose on the paleocerebellum the nature and intensity of the effort which must be accomplished, both in the somatic and in the autonomic sphere.

Summary

The author's previous experiments have shown that weak faradic stimulation of the vermian cortex of the anterior cerebellum causes strong inhibition, not only (as is well known) of decerebrate rigidity, but also of vasopressor and vasodilator reflexes and of the spontaneous vasomotor waves. The present paper concerns the action of the same cerebellar stimulation on vasomotor and respiratory carotid-sinus reflexes. The following effects have been observed:

1. Inhibition of carotid-sinus vasopressor reflexes brought about by the occlusion of the common carotid arteries. The depressor action on normal blood pressure is less evident or absent.

2. Inhibition of carotid-sinus respiratory reflexes produced by a carotid occlusion as above. There is also less evident inhibitory action on normal respiration.

3. Inhibition of the carotid-sinus reflexes, chiefly respiratory in nature provoked by an intracarotid injection of potassium cyanide.

Experiments of local cocainization, total or partial curarization and occasional observations show that the vegetative effects observed are (like the somatic ones) due to a central inhibition of bulbopontine centers by the paleocerebellar cortex. We must, therefore, admit the existence of a cerebellar control not only in the somatic but also in the vegetative sphere. The mechanism and the functional significance of the vegetative action of the paleocerebellum are discussed.

I am deeply indebted to Professor Frédéric Bremer, in whose laboratory this work was done, for his stimulating suggestions and criticisms. I wish to acknowledge the technical assistance of Mr. L. Chantraine.

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BRAIN POTENTIAL CHANGES IN MAN DURING CYCLOPROPANE ANESTHESIA

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IN AN earlier paper (Rubin and Freeman, 1938) it was reported that the intravenous injection of sodium cyanide resulted in the appearance of rhythmic 4 to 6 per sec. waves in the electroencephalograms of a narcoleptic individual and a stuporous catatonic patient. We suggested that this atypical cyanide response was due to a state of functional depression of cortical neurons. To elucidate the mechanisms underlying the production of slow potential changes, we have investigated the influence of cyclopropane anesthesia on brain wave frequencies. Since no systematic study of human brain potential changes in relation to anesthesia have been reported, this study has filled the gap in our knowledge. In addition, it provided the basis for interpretation of alterations in the electroencephalogram resulting from the injection of sodium cyanide at various levels of anesthesia, which will be reported in detail elsewhere.

METHODS

The patients were given a mixture of cyclopropane (trimethylene)* and oxygen to breathe. The induction period with this anesthetic is very short, the average time to reach surgical anesthesia being about 5 min. A total of 36 experiments were performed on 12 subjects, of whom 9 were schizophrenic patients and 3 were non-psychotic individuals. Since no differences were apparent in the data from the 2 groups, no distinction will be made in reporting the results of the experiments. The subjects had no breakfast and were given 1/100 grain of atropine subcutaneously about 15 minutes before anesthetization.

Brain potentials were recorded during each anesthesia period from monopolar disc-electrodes (solder) fixed to the scalp by a drop of collodion. The reference (earthed) lead was placed on the mastoid processes. Two independent, well-matched Grass amplifiers and ink-writing undulators were employed to record the electroencephalograms.

RESULTS

Anesthesia. During the course of cyclopropane anesthesia there are two distinct alterations of the electrical activity of the brain. After a minute or two, the alpha waves decrease in frequency by about 20 per cent and increase in amplitude by 100 per cent or more. (These 7 to 8 per sec. waves are abolished when the subject opens his eyes.) Occasional alpha waves of the original frequency are seen, and slower, random waves appear (Fig. 1, II). It is during the latter portion of this phase that the lid reflexes disappear. The same potential changes may be seen even in those individuals with no alpha activity in the waking state.

The second step is marked by a further increase in amplitude and by the

* We are grateful to E. R. Squibb and Sons for their generosity in supplying us with the cyclopropane.

appearance of rather regular 3 per sec. waves (Fig. 1, III). Again, as in the previous stage, occasional alpha waves of pre-anesthesia frequency are seen. The patient is now in surgical anesthesia, having breathed the anesthetic for 3 to 10 minutes in all. The only exception to this description is found in the case of the occipital lobes, where no regular frequency occurs, even in deepest anesthesia. We shall have more to say about this later.

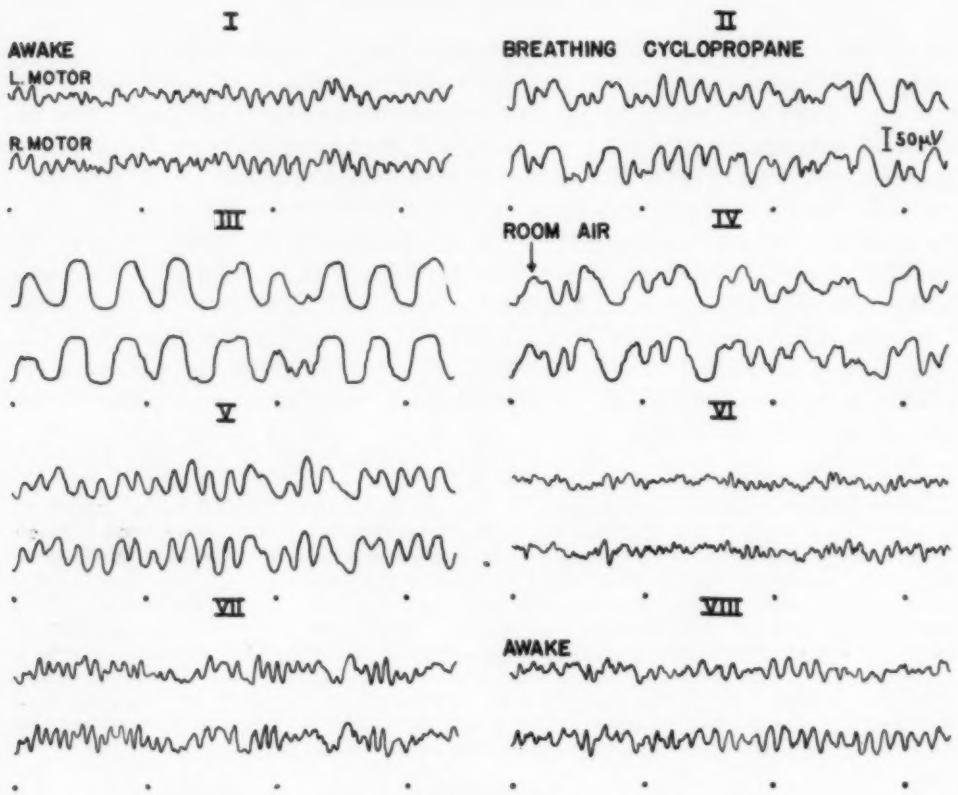

FIG. 1. Simultaneous records from the left and right motor regions, illustrating the changes in frequency during cyclopropane anesthesia and recovery. I, awake: II, breathing cyclopropane for 2 min.; III, 1 min. later; IV, immediately on substitution of room air for cyclopropane; V, 3 sec. later; VI, 2 min.; VII, 5 min.; VIII, completely recovered from the anesthesia. Amplification constant throughout. See text for further description.

Recovery from anesthesia. If the subject has not been kept in deep anesthesia for a great length of time (*i.e.*, more than 3 to 5 minutes), the early stages of recovery are extremely rapid. Immediately upon substituting room air for the cyclopropane mixture, irregular activity of lower voltage with mixed frequency appears (Fig. 1, IV). Isolated waves with durations of 1/30 to $\frac{1}{3}$ sec. are found in this stage.

On the other hand, when the patient is allowed to breathe room air after he has been in surgical anesthesia appreciably longer than 5 minutes, there is

a further slowing of frequency and the pattern is more irregular than that in deep anesthesia. This is presumably due to the increased absorption of accumulated cyclopropane which is occasioned by the change from shallow to deep respiration on breathing room air.

In the next stage, which follows the preceding one by a few seconds, there is an almost continuous 7 to 8 per sec. rhythm (Fig. 1, V). In the course of the next few minutes the amplitude drops progressively and the 7 to 8 per sec. waves disappear.

The third step in the recovery is marked by low-voltage (10μ V) potential changes of higher frequencies. The predominant frequency is 18 to 20 per sec., with occasional 10 per sec. and more numerous 12 to 14 per sec. waves interspersed (Fig. 1, VI). This stage may not occur in some subjects, in which case there is a very short period of relatively flat baseline with random waves of no fixed frequency.

In the last stage before complete recovery there is an increase in amplitude and a decrease in frequency, 12 to 14 per sec. waves predominating (Fig. 1, VII). In some subjects this rhythm may occur almost continuously for several minutes; in others it may appear in short bursts with relatively little activity in the intervals. In the next 5 or 10 minutes there is a gradual increase in the number of alpha waves, accompanied by a decrease in the number of 12 to 14 per sec. waves.

The subject may be easily aroused during this stage, as evidenced by his response to simple questions and by the sharp increase in the number of alpha waves appearing in the electroencephalogram. However, he soon slips back into a drowsy state, and if undisturbed will remain in this state for many minutes. Arousing the subject several times appreciably shortens the duration of this stage, so that he is fully conscious and exhibits the pattern of electrical activity characteristic for him in the waking state minutes sooner than would have been the case had he been left undisturbed. This is in direct contrast to the 14 per sec. stage in normal sleep, where the subject is in deep sleep and can be aroused only with great difficulty (Loomis *et al.*, 1937; Davis *et al.*, 1938).

Analysis of frequency changes. In describing the frequency changes in the previous section, predominating rhythms were employed as criteria. It is probably more significant to consider the shift which occurs during anesthesia in the frequency spectrum as a whole. This has been done in the following manner.

Eight consecutive seconds of record typical of each stage were selected and the duration of every potential change was measured with a millimeter rule. The speed of the recording tape was 30 mm. per sec., so that duration in mm. could be readily translated into fractions of seconds, and finally into number of waves per sec. Since this method is not sufficiently sensitive in detecting very small differences in wave duration, the data were treated as groups of frequencies. For example, a group with a range from 0.5 to 5 per sec. corresponds to delta activity; 8 to 10 per sec., in this individual, is the range of alpha-wave frequency; 10.5 to 15 per sec. includes the 12 to 14 per sec. waves characteristic of one of the stages of recovery from anesthesia, etc.

The number of waves (frequency of occurrence) of the various frequency groups in the 8-sec. sample were then plotted as a function of time (Fig. 2).

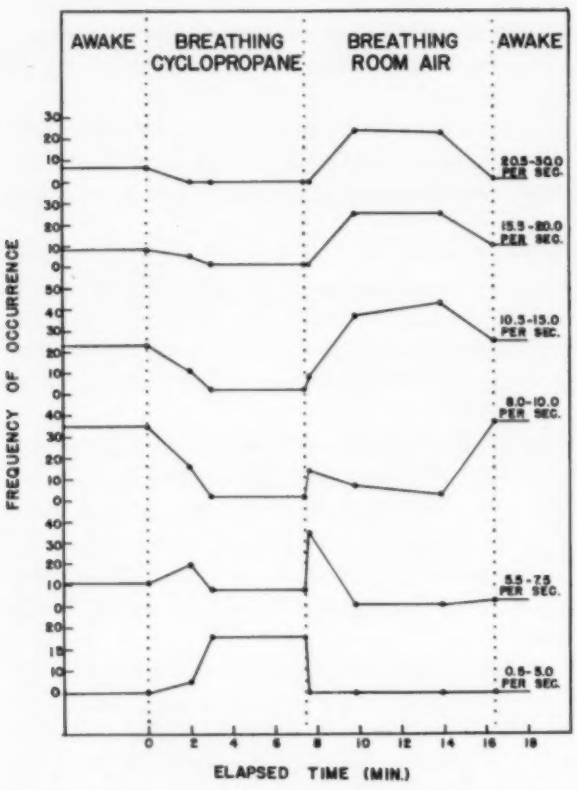

FIG. 2. A quantitative description of the number of waves of various frequencies observed in the several stages of a typical cyclopropane anesthesia.

It is interesting that the alpha rhythm is commonly present to some degree in its original frequency throughout the several stages of anesthesia and recovery from it.* In this particular case (Fig. 2) an exception to this statement is found in the stage of deepest anesthesia. It may be seen in Fig. 2 that the frequency changes in the approach to anesthesia are not mirrored in recovery from anesthesia. This has also been observed in normal sleep (Loomis, Harvey and Hobart, 1937; Blake, Gerard and Kleitman, 1939).

Architectonic distribution of frequency changes

A. The electrical patterns from corresponding regions of the two sides of the head during anesthesia and subsequent recovery from it are nearly superimposable (see Fig. 1). On the other hand, simultaneous tracings from the various architectonic regions of the cerebral cortex reveal marked differences which may be listed under 4 categories.

(i) *Time of onset of slow activity.* Slow potential changes appear earliest in the anesthesia in the frontal region. The motor and premotor regions are next, and the occipital and parietal regions the last to show 0.5 to 7.5 per sec. activity. The parietal is the most variable in this respect, sometimes being similar to the motor region and sometimes similar to the occiput.

Actually it is difficult to make a reliable generalization as to which regions manifest slow activity first. When differences in the time of onset of slow activity do occur, they are seldom greater than a few seconds. This may be due to the rapid rate of induction by cyclopropane, with the result that the separate phases of electrical activity associated with anesthesia are crowded together and not easily recognizable as discrete stages. Differences

* Alpha waves have also been reported during normal sleep (Blake and Gerard, 1937).

in time of onset of slow waves may be more clearly seen in sleep, where the diminution of consciousness is more gradual. For example, one of our patients became drowsy before the anesthetic was administered. In Fig. 3 are shown strips of record taken while the subject was drowsy and after he was awakened. A comparison of the tracings shows that the electrical activity of the parietal lobe was unaltered by the drowsiness, whereas the premotor region exhibited a regular 4 per sec. rhythm of increased voltage with alpha and 12 to 14 per sec. waves superimposed.

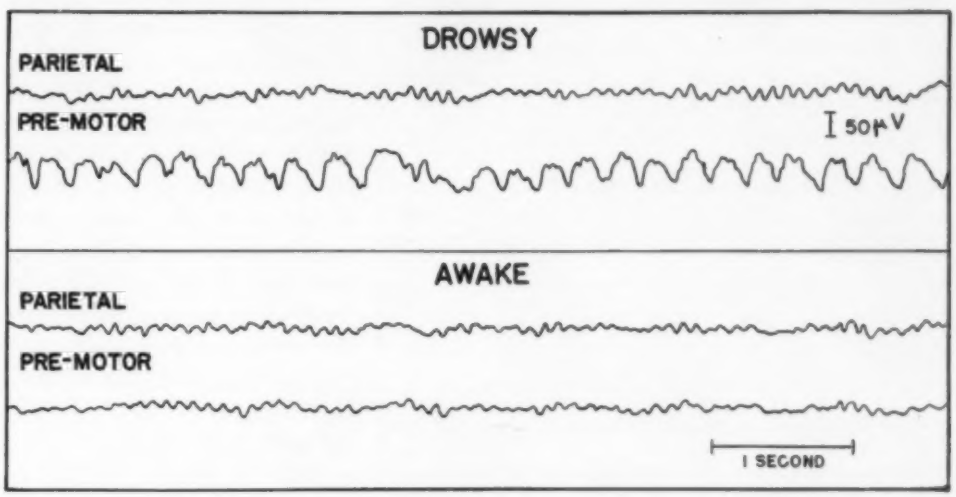

FIG. 3. Simultaneous records from the right parietal and premotor regions of a subject when drowsy and when subsequently awakened, illustrating the more rapid onset of slow potential changes in the premotor region and the independence of the 2 regions. Amplification the same in both records.

(ii) *Recovery rate.* Usually the motor and premotor regions are the first to recover from anesthesia. These two areas are then followed in recovery by the occipital and parietal regions. The frontal lobes are the last to show the waking potential pattern. The difference in recovery time may be as great as several minutes in some instances.

(iii) *Amplitude.* Amplitude of the slow waves during anesthesia is lowest in the occiput and increases progressively as one records anteriorly to the premotor region. Slow activity in the frontal lobes is as great as, and sometimes greater than, that in the motor and premotor regions.

(iv) *Regularity.* The most regular, slow potential changes are observed in the frontal lobes. This is less true of the areas posterior to the frontal region. Regular, slow rhythms are rarely seen in the occipital lobes, even in deepest anesthesia, although it is true that occipital delta waves may be of longer duration than those occurring elsewhere in the cortex.

Some of the preceding points are illustrated in Fig. 4. In addition, it illustrates another interesting point. During the latter part of the anesthesia,

the patient's respiration ceased, and it was necessary to employ artificial respiration. Almost all activity had disappeared from the occipital record, but there was an increase in frequency and a decrease in amplitude in the motor region (Fig. 4e). The patient was well saturated with cyclopropane at this time, and we do not believe that the anesthesia was lighter than previous to this incident.* In view of this, one might raise the question as to whether slow, regular potential changes are always a sign of depressed cortical activity, or whether they are merely indicative of enhanced synchronization which only certain conditions of cortical excitability make possible. Slow activity may well be a sign of *increased* cortical excitability, as we have pointed out elsewhere (Rubin and Freeman, 1938).

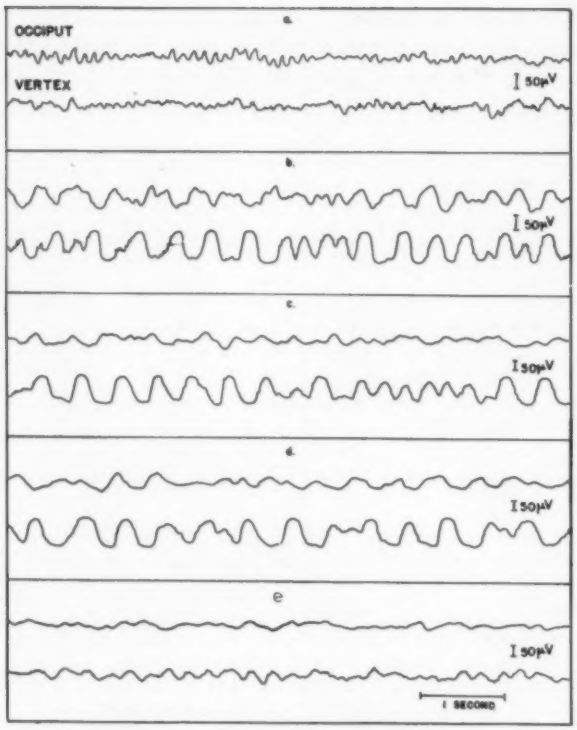

FIG. 4. Simultaneous records from the right occiput and vertex, *a*, awake; *b*, *c* and *d* during surgical anesthesia; *e*, during respiratory failure. Note the relative independence of the 2 regions, especially in *e*, and the increase in frequency in the vertex tracing in *e*.

The occipital electrogram during anesthesia is most readily distinguishable from that of other regions. Its lower amplitude and irregular potential pattern have already been mentioned. It is, in addition, quite variable. For example, in one subject irregular 5 to 6 per sec. activity was observed in the occipital record. Although this subject was kept under deep anesthesia for a considerably longer time than usual, there was no further decrease in frequency in the occiput. On another occasion, frequencies of 1 to 3 per sec. were seen in the occipital record of the same patient.

B. *The 12 to 14 per sec. rhythm.* Another feature which distinguishes the occipital region from the others is the infrequent occurrence of the 12 to 14 per sec. stage during recovery from anesthesia. When it does occur, the

* Irving (1938) has reported an increase in blood flow through the brain on cessation of ventilation of the lungs. The increased oxygen available to the brain and the facilitation of the removal of cyclopropane resulting from this might explain the frequency increase. However, the almost complete abolition, at this time, of activity from the occiput would argue against such an explanation.

rhythm is low in amplitude (very seldom above 20 μV) and appears in short, random bursts. The 12 to 14 per sec. stage is seen almost always in the motor and premotor regions, where it sometimes may occur as a more or less continuous discharge lasting for as long as a minute or more with an amplitude of 75 μV. This stage is not as marked in the frontal lobes.

C. *Synchronization.* With the exception of the occipital lobes, neighboring regions may exhibit such similar activity, especially in deep anesthesia, that their records are virtually superimposable. However, potential changes under electrodes as much as 10 or 12 cm. apart may also occasionally become synchronized. In the waking state the tracings from the premotor region and forehead are quite different (Fig. 5a). In deep anesthesia they are still clearly dissimilar (Fig. 5b). For brief intervals, however, the two regions may show good synchrony (Fig. 5c), then reverting to a state of pronounced asynchrony (Fig. 5d). This alternation may be repeated several times during the anesthesia, but never, in our experience, are the periods of synchrony of more than a few seconds in duration.

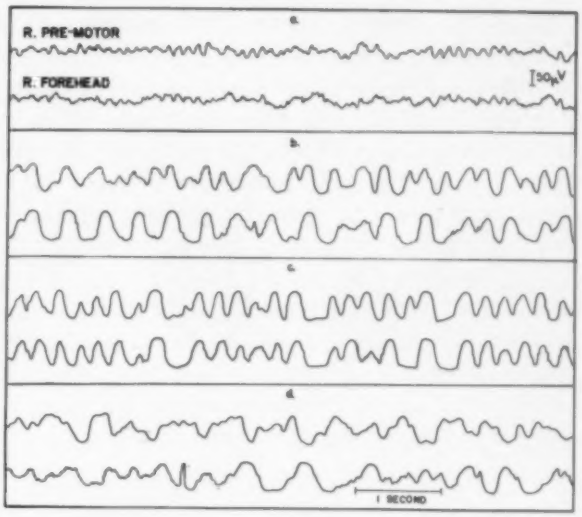

FIG. 5. Simultaneous records from premotor region and forehead, illustrating asynchrony in *a* (awake) and *b* (anesthesia); synchrony in *c;* and reversion to asynchrony in *d*. *b*, *c* and *d* taken during the same anesthesia. Amplification constant throughout.

In general, the cortex seems to act in 3 units as far as similarity in potential changes is concerned. During anesthesia and recovery from it, the activity is more or less the same throughout the frontal region; the motor and premotor regions form another unit; and, finally, the parietal and occipital areas form a third unit. There are exceptions to this, in that the parietal lobes may, in some instances, show the same electrical activity as the motor and premotor regions, and, less frequently, the frontal and premotor regions may beat in unison. These observations are in harmony with the view that the various cortical regions in man are relatively independent of each other in the production of their electrical activity Jasper and Andrews, 1938; Rubin, 1938). In their study of the distribution of potential changes during normal sleep, Loomis, Harvey and Hobart (1938) report that right and left halves of the head show similar patterns while front, top

and back may be quite different. The distribution during cyclopropane anesthesia, therefore, is essentially the same as that in normal sleep.

Variability. The course of anesthesia is not a smooth one, as judged by the observed potential changes. Frequent fluctuations between successive stages are seen superimposed upon a more gradual, unidirectional trend. This is especially true in the later stages of recovery from anesthesia.

There is a remarkable constancy of the potential patterns which a given individual exhibits in repeated anesthesias on the same day or from day to day. Although there is less differentiation between individuals in anesthesia than in the waking state, there are certain distinguishing characteristics. For example, some subjects may be brought to surgical anesthesia in 3 minutes or less, whereas it takes 10 minutes or longer for others. During surgical anesthesia the frequency may go as low as 0.5 to 1 per sec. in some individuals, but in others the same region may never show a rhythm slower than 4 to 6 per sec. The most common frequency we observed in deep anesthesia was 3 per sec. By utilizing a variety of criteria of this sort, one can distinguish between individuals with a fair degree of accuracy.

Influence of CO_2. In the majority of the experiments the CO_2 exhaled by the patient was absorbed by soda lime. However, in some instances it was necessary to allow the patient to rebreathe his own CO_2 at an early point in the anesthesia to prevent respiratory failure. This always hastened the onset of deep anesthesia and made the slow rhythms characteristic of this stage much more regular than usual.

Discussion

If the changes in frequency observed during cyclopropane anesthesia and recovery from it were due solely to a decrease in metabolism of neurons, we should expect them to be gradual and unidirectional (*i.e.*, either progressively increasing or decreasing in frequency as the case may be). Brain metabolism does decrease during anesthesia (Serota and Gerard, 1938). However, the decreases in frequency observed in the electrograms during anesthesia are quite abrupt. The series of frequency changes during recovery from anesthesia (*i.e.*, from 3 per sec.→mixed 3 to 30 per sec.→7 to 8 per sec.→18 to 20 per sec.→12 to 14 per sec.→10 per sec.) is even more difficult to explain on a metabolic basis. Undoubtedly other factors must be involved. For instance, cell permeability is decreased during anesthesia (Spiegel and Spiegel-Adolf, 1936). This, in turn, must influence the ionic relationship between the cells and their environment, which may be reflected in frequency and amplitude changes (Libet and Gerard, 1939). In addition, a blocking of afferent impulses may occur during anesthesia (Beecher, McDonough and Forbes, 1938). Such a blocking is effective in altering the frequency of cortical potentials (Bremer, 1935). All the preceding factors probably contribute in bringing cortical neurons to various levels of excitation at which synchronization can occur. Adrian (1937) has presented evidence that fixed rhythms can occur only when synchrony is good,

and that synchronization in a given group of neurons is a function of the frequency of discharge of individual neurons at a given moment. The present data seem to show several such excitation levels with rather sharply defined thresholds.

Although such a mechanism as just described would hold whether one assumed that different neurons or the same neurons were responsible for various frequencies, the evidence is definitely in favor of the latter view. Adrian's (1937) data indicate that the same neurons can discharge at many frequencies. Libet and Gerard (1938) have demonstrated that the same neurons in the isolated frog brain are capable of "beating" at frequencies from 1 to 50 per sec. The appearance of many distinct frequencies over a period of a few minutes after prolonged insomnia (Blake, Gerard, and Kleitman, 1939) and our present observations of abrupt frequency changes during cyclopropane anesthesia are also in accord with this point of view.

Summary

1. There are 6 discrete changes in the frequency of brain potentials in man during cyclopropane anesthesia and recovery from it. The frequency changes in the approach to anesthesia are not mirrored in the recovery from anesthesia.

2. Potential patterns from corresponding regions of the head during anesthesia and recovery are almost identical. However, the front, top and back of the head form 3 relatively independent units of activity. Differences in time of onset of slow activity, rate of recovery, amplitude and regularity of potentials in the 3 units are described.

3. Just as in normal sleep, a well defined 12 to 14 per sec. rhythm is found most predominantly on top, less so in front and least commonly in the back of the head. This rhythm differs from that in normal sleep in that it appears late in the recovery from the anesthetic.

4. Although there is not as great individuality of potential patterns during anesthesia as in the waking state, it is nevertheless possible to differentiate between anesthetized individuals on the basis of their electroencephalograms.

5. CO_2 hastens the onset of deep anesthesia and makes slow rhythms more regular.

6. Factors which may contribute to the frequency changes seen in cyclopropane anesthesia are discussed.

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CONDITIONED VESTIBULAR REACTIONS

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THE EXPERIMENTS described here arose from interest in the possibility of using vestibular responses as a basis on which to form conditioned reflexes, and in the pathways involved in their establishment. This problem grew out of recent work of this laboratory in: (i) describing the type of physiological reactions which can be conditioned and (ii) delimiting the elements of the segmental and suprasegmental structures essential in the formation of the conditioned response (Gantt 1937a and b; Brogden and Gantt 1937).

In spite of numerous physiological and anatomical studies on this question our knowledge of the representation of the vestibular apparatus in the cortex is still meager. Furthermore certain mental diseases show changes in the irritability of the vestibular apparatus which seem to be related to the irritability of the cortex in general (Aubry and Baruk 1929; Joo and Meduna 1935; Löwenbach 1936). Here also nothing is known of the pathways and centers having to do with the disturbance.

METHODS

Of the three methods of producing vestibular reactions: (i) acceleration, (ii) caloric irritation, (iii) galvanic stimulation, we have used the third because of its ease of application in animals. We were fully aware that this stimulus was the most "inadequate" of the three and had some other disadvantages. The galvanic reactions are produced not by direct stimulation of the semicircular canals but by stimulation of the vestibular ganglion (Dohlman 1926, 1929). The subjective feelings, as known from experiments in man, begin a few seconds after the make of the current. As we used the slightest movement of the head as an indicator of vestibular reaction, and as we know from the work of Bourguignon (1927) and others that such movements follow almost immediately upon the stimulation, the delayed appearance of subjective feelings was of no significance for our experiments. The fact that the break of the current constitutes an additional stimulus is another obstacle. We tried first to overcome this difficulty by not breaking the current abruptly, but decreasing it gradually for a longer period of time. However, this did not abolish the stimulating effect. The experiments later demonstrated that in the dogs which were used, the make of the current was most significant and after a short while the animals neglected the break stimulus. Electrodes made out of copper wire thickly covered with gauze and soaked in saline solution were introduced into both external auditory meatuses, held in their place by a bandage, and the animals blindfolded. The electrodes were connected with a 15 volt battery through a potentiometer and currents between 0.5 and 10 milliamps. applied (unconditioned stimulus). The conditioned stimuli were a bell, a tone, or a buzzer acting for 1 to 3 seconds before the onset of the galvanic shock and continued 1 or 2 seconds after the shock.

RESULTS

The elaboration of conditioned responses was attempted in four dogs. *Vera*, a placid, quiet and phlegmatic female about 12 years old, had been used several years previously for the study of conditioned food responses. *Vespasian*, a male hound about 3 or 4 years old, within the past year had

elaborated conditioned defense reactions to an electric shock. *Vestus* and *Vesal*, had been used similarly to Vespasian.

The unconditioned reflex to the galvanic shock consists in an initial jerk of the head toward the side of the positive electrode, a lowering of the head so that the nose frequently touches the floor and elevation of the negative ear to as much as 90 degrees, a change in muscle tonus on the two sides so that the animal resists having his head or his body pushed to the negative side, and sometimes bending of the body to the positive side and going around in circles toward the anode. Most constant of these reactions was the elevation of the ear and the turning of the snout to one side. Occasionally the dog made side-stepping movements or even fell over. Symptoms of nausea, licking out the tongue, and retraction of the lips sometimes appeared. The positive conditioned response was recorded if one or more of the above components of the unconditioned reactions were observed.

The first conditioned reactions were those of a defense nature, mild to violent struggling according to the temperament of the dog, trying to remove with the forefeet the electrodes from the ears or the bandage from the head. As the conditioned responses changed from defense to specific reactions based on the pattern of the unconditioned reflex, the dog began at first to sway from one side to the other. This swaying accompanied a change of muscle tonus on the two sides. Gradually various elements seen in the unconditioned reflexes appeared as components of the conditioned responses.

After the full development of the specific conditioned response, differentiation was tried in two dogs. In order to accomplish differentiation the direction of the current was reversed, so that the movements of an opposite nature were elicited by this reversed current (elevation of the other ear, movement of the head to the opposite side, circular movements counterclockwise instead of clockwise, increased muscle tonus on the opposite side, etc.). A new conditioned stimulus was used for the establishment of the new conditioned response. After a certain number of trials the new conditioned stimulus produced movements similar to the new unconditioned reflexes. During the early use of the new signal it elicited the same conditioned responses as the first conditioned signal had, as is generally the case until a differentiation is firmly established.

Vera had been used in 1936 for the establishment of conditioned responses both to food and to acid. These were readily formed after 10 to 15 trials. Experiments on the vestibular reflexes began on the 15th of January 1939. The animal was used in the camera where the food reflexes were previously established. After the 10th trial, using the tone as the conditioned signal for the vestibular reflexes, the animal no longer turned to the food box but began to give specific conditioned vestibular reactions. This at first consisted in turning the head slightly to the left (same side as unconditioned vestibular reflex but opposite to former food conditioned reflex). The latent period was short—about ½ to 1 second. This component, swaying and turning the head to the left, continued after the 11th combination with oc-

casional failures. Later an elevation of the right ear and lowering of the head was added, being constant after about the 100th trial. These reactions were regular and constant until the 211th trial on February 21st, 1939. Vera became very quiet and gave practically no other movements than the specific conditioned responses and the unconditioned reflexes to which latter were occasionally added slight whining after the shock. On the 20th of April differentiation was begun. Retention of the conditioned reflex to the tone was perfect for this two month rest period. A buzzer was used as the new conditioned signal for the reversed current. For the first six trials Vera gave the same conditioned responses to the buzzer as she had to the tone, opposite to those of the new unconditioned reflex, but beginning with the 7th repetition of the buzzer she began to give the appropriate new conditioned reflex —movement of the head to the right, turning to the right, with continued lowering of the head. Elevation of the left ear began only on the 37th repetition of the buzzer and continued thereafter infrequently; the elevation of the right ear completely disappeared after the 34th repetition of the buzzer. The head was bent to the right 15 to 60 degrees. An indifferent sound, a bell, was used to test for generalization; there was absolutely no reaction.

Vespasian had been used several months previously in experiments in which a bell was the signal for a shock on the thorax, the conditioned response being raising of the right foot. On February 21st, 1939, vestibular conditioning was started. At this time the muscular tonus was the same on the two sides. When blindfolded and prepared for the experiment he tucked his tail but remained quiescent. A tone of 1000 c./sec. was the conditioned signal, followed in three seconds by the unconditioned stimulus—a current of 2 to 10 mA, the right ear being positive. There was no reaction to the tone when first used. This dog like Vera showed little defense. The unconditioned reflex consisted in rotating the head so that the left ear was uppermost, occasionally falling over, stepping to the right and running around in circles to the right. Later, the head was lowered so that it was pressed flat on the floor, the right ear being often brought in contact with the floor. With the strong current there was occasional whining.

The conditioned reflex was first seen on the 8th repetition of the tone, consisting of a running to the right and a slight rotation of the head with the left ear up. The movements of the conditioned reflex fluctuated from the 8th to the 72nd repetition; during this time the dog went to the left as often as to the right. There was often a slight swaying of the head from side to side. The presence of nausea was suggested by marked salivation, labored breathing, and lowered head, but this was always transient and the dog readily ate as soon as the bandage was removed from the eyes. The conditioned reflex became stable after the 75th trial on the 25th of February, and was often more marked than the unconditioned reflex. As it became more stereotyped the dog would lower the head nearly to the floor or even on the floor with an elevation of the left ear, and sometimes there was a loss of balance and falling during the conditioned reflex. A posture with the head

rotated, the left ear always being uppermost, was often assumed in the intervals. Fewer extraneous movements occurred so that there was no struggling and only the conditioned reflexes were present. A bell introduced after the 149th repetition of the tone produced no response, showing that there was no generalization. After the tone had been given for 179 times, the buzzer was used for differentiation. During the first trial there was a conditioned reflex to the buzzer. For the purpose of differentiation the electrode in the right ear was changed from positive to negative, thus producing an unconditioned reflex of opposite nature—rotation of the head with *right* ear up, turning of the head toward the *left* and going toward the *left*. Occasionally instead of this appropriate unconditioned reflex the former one appeared.

After the fourth repetition of the buzzer a conditioned reflex occurred, but instead of being equivalent to the new unconditioned reflex it was identical with the conditioned reflex to the tone. Differentiation took place in stages. The turning of the animal to the left instead of to the right, first seen on the 8th repetition of the buzzer, was not constant until much later—after the 250th trial. The rotation of the head in the proper direction, right ear uppermost, began to supplant the old movement of left ear up on 38th repetition. For a long time differentiation was imperfect and until the 237th repetition about one half of the time the head would be turned to the right as it had been with the tone. Later the new conditioned reflex became constant. Conditioned turning of the whole body to the left—a component of the new unconditioned reflex—appeared first on the 189th trial of the tone and occurred fairly frequently after the 248th repetition.

Very striking in this dog, and in this dog only, were the *accessory motor phenomena*, which became prominent during the differentiation period. The postures resembled those described by Pavlov as hypnotic and cataleptic but were even more marked. At times the animal became practically non-reactive to many diverse external stimuli as pinching, loud noises, or needle-pricking, but paradoxically he was nearly always reactive to the conditioned stimulus though apparently asleep. A peculiar position was often assumed for long periods. Sometimes the head was lowered to the floor in a praying attitude, sometimes the body was curved into a complete circle so that the snout touched the hip, at other times the dog curled on the floor, breathed slowly and snored. Such an apparent sleep would last for as long as 15 minutes during which the animal was non-reactive except to the conditioned stimulus, to which he would usually give a slight response. Although some of these reactions were first seen on February 1, the sleep and snoring did not become prominent until differentiation was introduced on March 20. During such sleep the respiration rate was 9 per minute and the heart rate 55–65 compared with 85 at the instant of awaking and 110–120 while eating. The dog could be put into various positions, all of them very unnatural for a dog, without resisting. The knee-jerk was very active during sleep. He did not notice food placed under his nose, although he readily ate it as soon as bandage and electrodes were removed. He was non-reactive to pricking with

hypodermic needles in the legs and over the scalp and also touching the penis, which was frequently partially erected. His limbs were more flaccid than rigid. The appearance of this sleep became routine during differentiation, but gradually became less frequent after the 150th repetition of the buzzer. The conditioned reflex during sleep consisted usually in only a slight rotation of the head.

The development of the conditioned vestibular reflex occurred without difficulty in the two other dogs. Differentiation was not tried in these dogs.

Tested for retention after a five month rest interval in Vespasian and an eight month interval in Vera they both showed perfect retention of the positive vestibular conditioned reflexes to the buzzer. This compares favorably with the retention of food conditioned reflexes, indicating the stability of the vestibular conditioned reflexes.

Discussion

This study establishes the fact that definite and specific vestibular reflexes can be conditioned. The second interest as stated above, concerning the pathways involved, is to be the subject of further investigation. The responses which we conditioned were so similar to the unconditioned vestibular reflexes produced by the galvanic current—often even much more pronounced—that we feel justified in concluding that they were specific vestibular reactions without admixture of defense reflexes. Most of the dogs gave practically no defense reflexes, except for an occasional slight whine, after the first few days of experimentation. The specificity of the conditioned reflex elaborated is further proven by the ability of the animal to differentiate, thereby giving specific movements in the opposite direction to those previously elaborated in the first stage of experimentation. That several elements of the unconditioned reflex were so exactly duplicated in the conditioned reflex is evidence against the argument that the animal might be making conditioned orienting reactions based on the tactile stimulation necessarily accompanying the make of the current.

There are important reasons why the reactions which we conditioned cannot be considered as arising from proprioceptive sensations following the unconditioned movement of the head. First, our reactions occurred very quickly, while proprioceptive conditioned reflexes are formed, if at all, only after hundreds of repetitions. Furthermore, in experiments performed previously in this laboratory, a movement produced by direct stimulation of the motor area of the cortex could not be conditioned.

The method employed to evoke the unconditioned reflex had, as stated in the beginning, the disadvantage of producing with the break of the current a separate complex of vestibular reactions opposite to the reactions to the make of the current. This disadvantage, however, gave further proof of the specificity of the reactions. After a very short period, when the dogs obviously were confused and did not give clear-cut responses to either the make or the break of the current, they learned to relate the tone or the buzzer only

to the make, and the conditioned reflex was then quickly established. Sometimes the dogs, after they had already given the proper conditioned reflex to the conditioned signal and received the reinforcement with the make of the current, would turn their heads to the opposite direction shortly before the current was broken again. In such a manner they showed a conditioning also to the break of the current.

The vestibular reactions, while not clearly autonomic, are closely allied to certain cerebellar reflexes, *e.g.*, those involving "tonus" and equilibration (Clark, 1939). Both vestibular and cerebellar reflexes are generally considered as "involuntary" movements, such as the withdrawal of the paw from a painful stimulus. Also there does not seem to be any essential difference in differentiation and retention of these types of reflexes. Other components of vestibular responses, as nystagmus, nausea, etc., and conditioning to "adequate" stimuli, are the subjects of investigations in progress.

Summary

In four dogs, using a galvanic current between the external auditory meatuses as unconditioned stimulus to produce vestibular reflexes (loss of balance, falling to one side, characteristic head and body movements), these reflexes readily appeared as conditioned responses to an auditory stimulus. Differentiation in these animals was also attained (by reversing the current to produce opposite movements which became conditioned to a new auditory stimulus). Prolonged sleep and peculiar motor phenomena developed in one dog. The conditioned vestibular responses persisted without practise for at least eight months.

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MODIFICATION OF THE CORTICAL FREQUENCY SPECTRUM BY CHANGES IN CO_2, BLOOD SUGAR, AND O_2*

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MANY WORKERS are now agreed that electrical activity of the cortex is greatly modified by changes in blood sugar (1, 2, 3, 4, 5, 6, 7, 8), by changes in blood O_2 (5, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18), and by changes in blood CO_2 (5, 12, 13, 14, 15). There is some dispute, however, about the exact nature of these modifications and their possible quantitative relation to any given blood constituent. Data from all previous studies have been based on a visual analysis of the electroencephalogram. Such an analysis is necessarily highly subjective. Changes in frequency which may appear sufficiently obvious to one observer to be easily quantitated by counting and averaging wave lengths, may not appear at all obvious to another. A purely objective method of analysis has been devised recently by A. M. Grass (20). We have in this study used the Grass method of analysis for extending the work of Lennox, Gibbs and Gibbs (5) on the alteration in electrical activity of the cortex with changes in various normal blood borne constituents.

METHODS

Grass method for cortical frequency spectra.—The electroencephalogram is taken as a shadowgram on film. Any desired sample of the record is made into a continuous belt and rotated between a light and a photoelectric cell. The resulting fluctuations in potential in the cell are amplified and passed through an exceedingly sharp continuously variable filter, which is connected with a recording galvanometer. By this means, it is possible to record automatically the amount of energy in all frequencies over a wide range. The expression which one obtains is a spectrum, or more accurately, the Fourier transform of the electroencephalogram. It is the most adequate compact expression obtainable for this type of record.

The value of such an expression was appreciated by Dietsch (21) who in 1932 made a harmonic analysis of the electroencephalogram. His figures do not give continuous spectra but represent only 18 points in the spectrum. Although his data in each case were obtained from measurements on only a few waves, each analysis required many hours of careful measurement. Similar mathematical analysis of a few waves was carried out by Rohracher (22) who used 24 ordinates. That these studies are not more significant is due to the inadequacy of the sample used and to the fact that despite much labor, the spectrum obtained is non-continuous. With the Grass method of analysis, in five minutes one can obtain a continuous spectrum of the energy in all frequencies from 1 to 60 per sec. in a strip of record 30 sec. in duration. The electroencephalogram which was analyzed was in all cases, that obtained with a "stigmatic" electrode on the scalp in the left occipital area and "indifferent" electrodes on both ears.

Method of studying blood constituents.—The chief source of error in the present study

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does not lie in the recording nor in the analyzing of cortical activity but in the proper quantitation of the blood constituents which alter the activity of the cortex. Lennox and E. L. Gibbs (25), Gibbs, Gibbs and Lennox (26), and Gibbs (5) have shown that respired air, arterial blood, and venous blood from the arm or leg are poor indicators of the CO_2 tension in the brain because the cerebral blood vessels are actively engaged in maintaining the CO_2 tension of the brain relatively constant despite changes in the CO_2 tension of the arterial blood. When the CO_2 in the artery falls, the cerebral blood vessels constrict reducing cerebral blood flow and so conserving CO_2 in the brain, while the limb vessels dilate, allowing CO_2 to be washed out of the limbs. With the CO_2 from the limbs swept into the general circulation, the drop in arterial CO_2 is somewhat checked and the cerebral blood vessels need not constrict so far in order to keep the CO_2 tension of the cerebral blood relatively constant. The constriction of the cerebral vessels with low CO_2 however, becomes less effective as the brain approaches anoxia. To be more specific, when the venous blood returning from the brain is 40 per cent saturated with O_2 instead of 50 to 60 per cent saturated as it is normally, the constrictor effect to low CO_2 begins to fail: When the venous blood from the brain is 28 per cent saturated with O_2, the point at which consciousness is lost, the constrictor effect to low CO_2 disappears. This failure of the constrictor effect of low CO_2 is in large part responsible for the dilatation which accompanies low O_2. Added to this, however, there appears to be a direct dilator effect of low O_2 on the cerebral vessels. No change in cerebral blood flow has been detected as a result of varying the blood sugar level.

In order to get close to the nerve cell environment, we have taken blood from high up in the internal jugular vein. It would have been better perhaps if data could have been obtained on the tissue fluid of the brain directly under the "stigmatic" electrode, as has been done by Dusser de Barenne, McCulloch and Nims (19). But what we have lost in directness, is partly made up for by the certainty that the technique we have employed could not possibly have injured normal responsiveness of blood vessels or nerve cells. Blood from the jugular vein at a point just below the jugular bulb is under ordinary conditions in equilibrium with the tissue fluids of the brain. It should reflect fairly accurately all gradual changes in diffusible substances. Sudden changes, however, will not be accurately reflected. In order to avoid error due to lag, all changes in blood constituents were made slowly and samples were taken when it was believed that equilibrium had been established. All CO_2 and O_2 determinations were done by the Van Slyke manometric method, sugar determinations were done by the Folin-Wu method.

Material—All subjects were adult men whose brains were normal. In the studies on high O_2 pressure, however, rabbits were used because the high O_2 pressure necessary to produce marked changes in the cortical frequency spectra are so high that they are difficult to maintain and properly control for an animal as large as man. The cerebral venous blood was not studied in these high O_2 experiments. It seemed reasonable to assume that the increase in O_2 pressure in the arterial blood, which occurs with an increase in atmospheric pressure, is due to an increase in the amount of O_2 in simple solution. Thus, the rise in O_2 pressure in the arterial blood will be a direct function of the partial pressure of O_2 in the atmosphere. Nevertheless it is hazardous to use the O_2 pressure in the arterial blood as an indicator of the O_2 pressure in the brain. The technical difficulty of taking blood samples under such high pressures caused us to proceed without determining the O_2 content of the cerebral venous blood, especially as we were not so much interested in the exact O_2 pressure at which changes in the cortical frequency spectrum occur, as in establishing the fact that certain changes do occur and that they occur at a critically high O_2 pressure.

The studies on high and low sugar were done on three normal subjects, those on high and low CO_2 on four normal subjects and those on low O_2 on three normal subjects. The data on high O_2 are based on experiments on four rabbits.

Results

As will be seen in Fig. 1 to 4, slight changes in the CO_2 content of the blood in the internal jugular vein and presumably in the CO_2 tension of the brain are associated with marked changes in the cortical frequency spectrum. These can be described most accurately by saying that as the CO_2 falls,

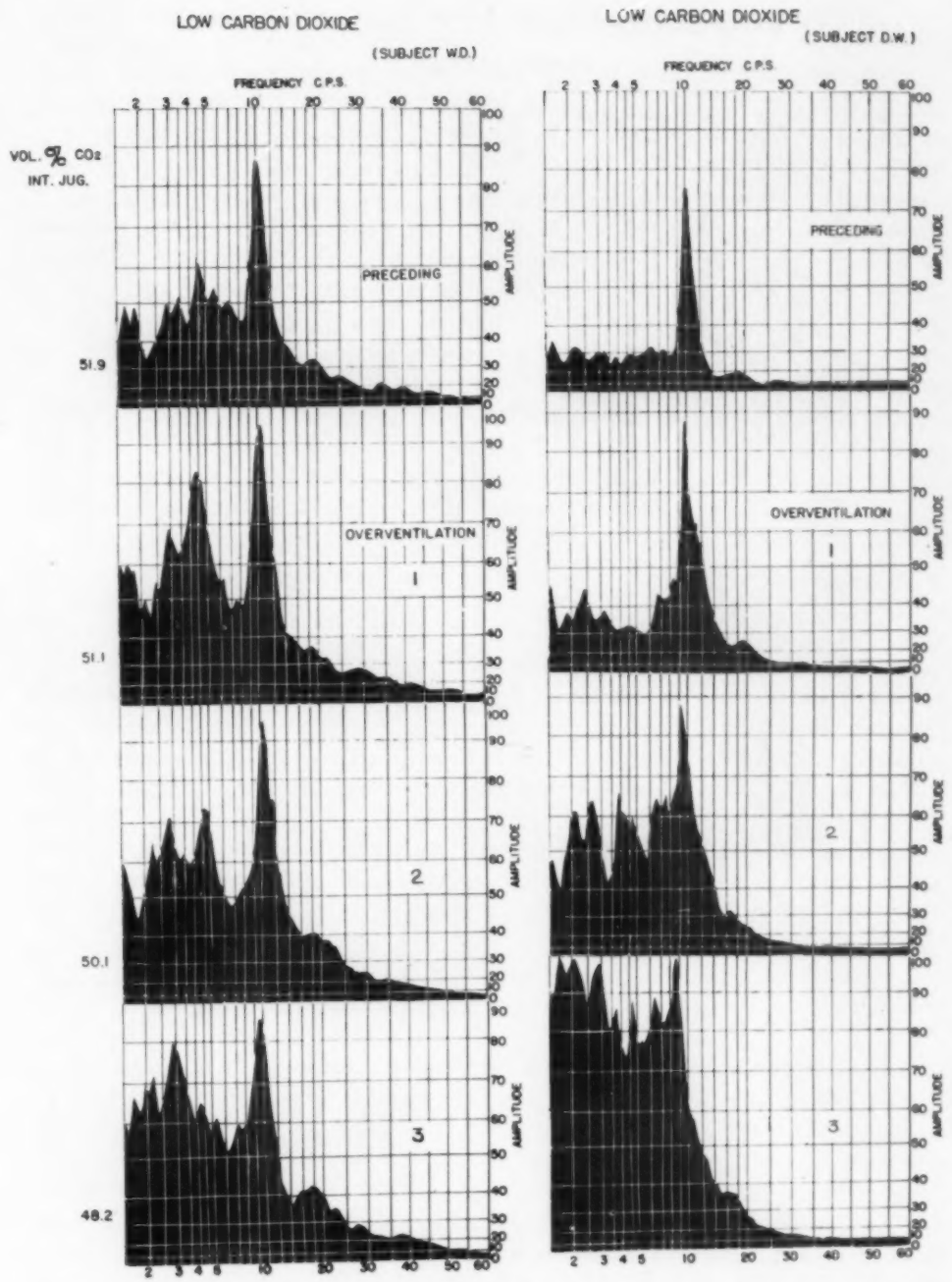

FIG. 1 (*Left*). A shift of the 19-per-sec. peak to the 18-per-sec. position, and a shift of the 4.5-per-sec. peak toward 4 occurs in this subject with a fall of the CO_2 content of the internal jugular blood from 51.9 to 51.1 volumes per cent. His 10-per-sec. peak is extraordinarily stable. It shifts perceptibly, however, toward 9.5 when the CO_2 content falls to 48.2. With the movement of energy toward the slow side, there is an increase in the total amount of energy in the spectrum.

FIG. 2 (*Right*). The 10-per-sec. peak in this subject is quite labile, moving further and further to the slow side as the CO_2 is decreased. No blood samples were taken.

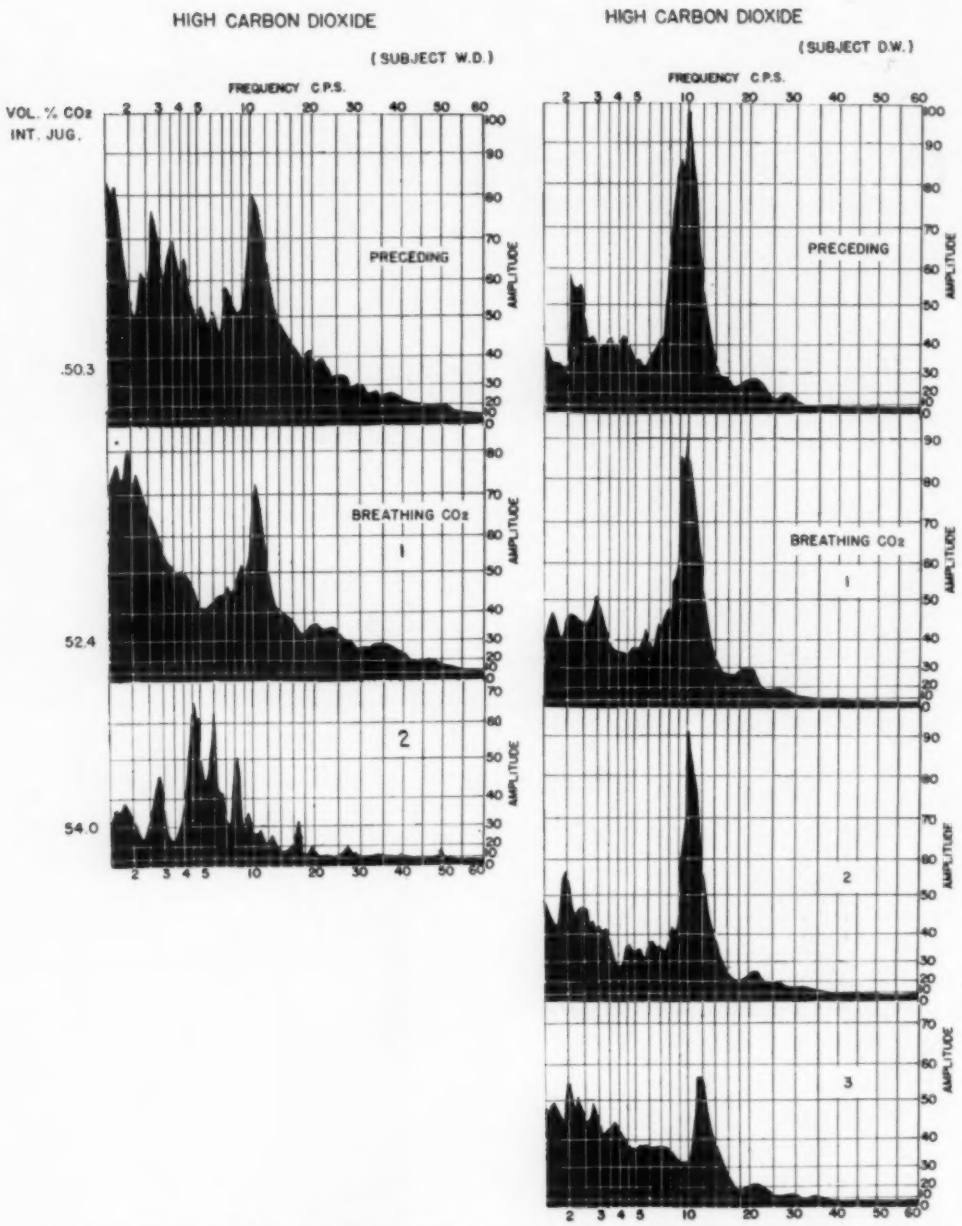

FIG. 3 (*Left*). The change in the CO_2 content of internal jugular blood from 50.3 to 52.4 is associated in this case with a shift of the 19-per-sec. peak to the 20-per-sec. position the 10-per-sec. peak shifts toward 11, and the peak below 2-per-sec. to a position very close to 2. There is also a relative increase in the energy between 25 and 35 per sec. With a CO_2 content of 54.0, the maximum near 2 becomes lower than the maximum at 2.8. The complex seen at 4, 5.5 and 8 resembles that seen at 2.7, 3.5 and 4 in the first of this series of spectra. The ten peak has disappeared and a definite peak has appeared at 17 per sec. The energy in the spectrum has been decreasing and has shifted toward the fast side. Its maximum has moved from below 2 per second to 2 per sec. and finally to 4 per sec.

FIG. 4 (*Right*). The gradual shift in this case of the 10-per-sec. peak toward the fast side as the CO_2 is increased is clearly illustrated. No blood samples were taken.

there is a shift in energy toward the slow side of the spectrum with an apparent increase in the total amount of energy. An increase in CO_2 is associated with a shift in energy toward the fast side of the spectrum with an apparent decrease in the total amount of energy. With low O_2 (Fig. 5), there is little change in the cortical frequency spectrum until a level of 40 per cent O_2 saturation in the internal jugular vein is reached, when there is a shift in energy toward the slow side with an apparent increase quickly followed by an apparent decrease in total energy. With high O_2 (Fig. 6), there is little change until an O_2 pressure approximately 35 lbs. per square inch is reached; at this point there is a sudden shift toward the fast side of the spectrum with an apparent increase in the total amount of energy. Low sugar (Fig. 7) produces little change until the blood in the internal jugular vein reaches a level of 30 mg. per 100 cc., when there is a sudden shift toward the slow side with an apparent decrease in the total amount of energy. When the blood sugar is raised, a change occurs which is similar to that seen with high O_2. At a blood sugar level of 500 mg. per 100 cc., there is an abrupt shift of energy toward the fast side of the spectrum with an apparent increase in the total amount of energy.

Discussion

The level of CO_2 in the internal jugular vein is the only factor which has a definite relationship to the changes which occur in cortical frequency when one overventilates or breathes high CO_2. It is not known whether the effect of changes in CO_2 tension on cortical cells is direct or indirect. It may be due to specialized chemo-receptors which indirectly alter the cortical activity. A mechanism similar to that found in the respiratory center, with its specialized accessory chemo-receptor, the carotid sinus, may be involved. If so, the chemo-receptor in this case must lie somewhere in the brain. That certain elements are more sensitive than others to CO_2, is altogether likely. Such a specialized chemo-receptor has not been demonstrated, however, so that it is wiser to consider that CO_2 has its effect directly on the cortical cells.

Other workers have spoken of the response of cortical potentials to a decrease in CO_2 as an "increase in activity," but this term is not sufficiently specific to describe what occurs. Nor is it possible to describe these changes in terms of amplitude only, as for example by saying that low CO_2 produces an increase in amplitude. The truth or falsity of this statement will depend upon what frequencies are referred to and the particular level of the CO_2 that is meant. The amount of 10-per-sec. activity in subjects D.W. and W.D. (Fig. 1 and 2) first increases and then decreases as the CO_2 level continues to fall.

The frequencies at which peaks or maxima appear at the fast end of the spectrum are often multiples or near multiples of the frequencies at which peaks appear at the slow end of the spectrum. These peaks may shift independently of one another. A slow peak may disappear, and the peak at

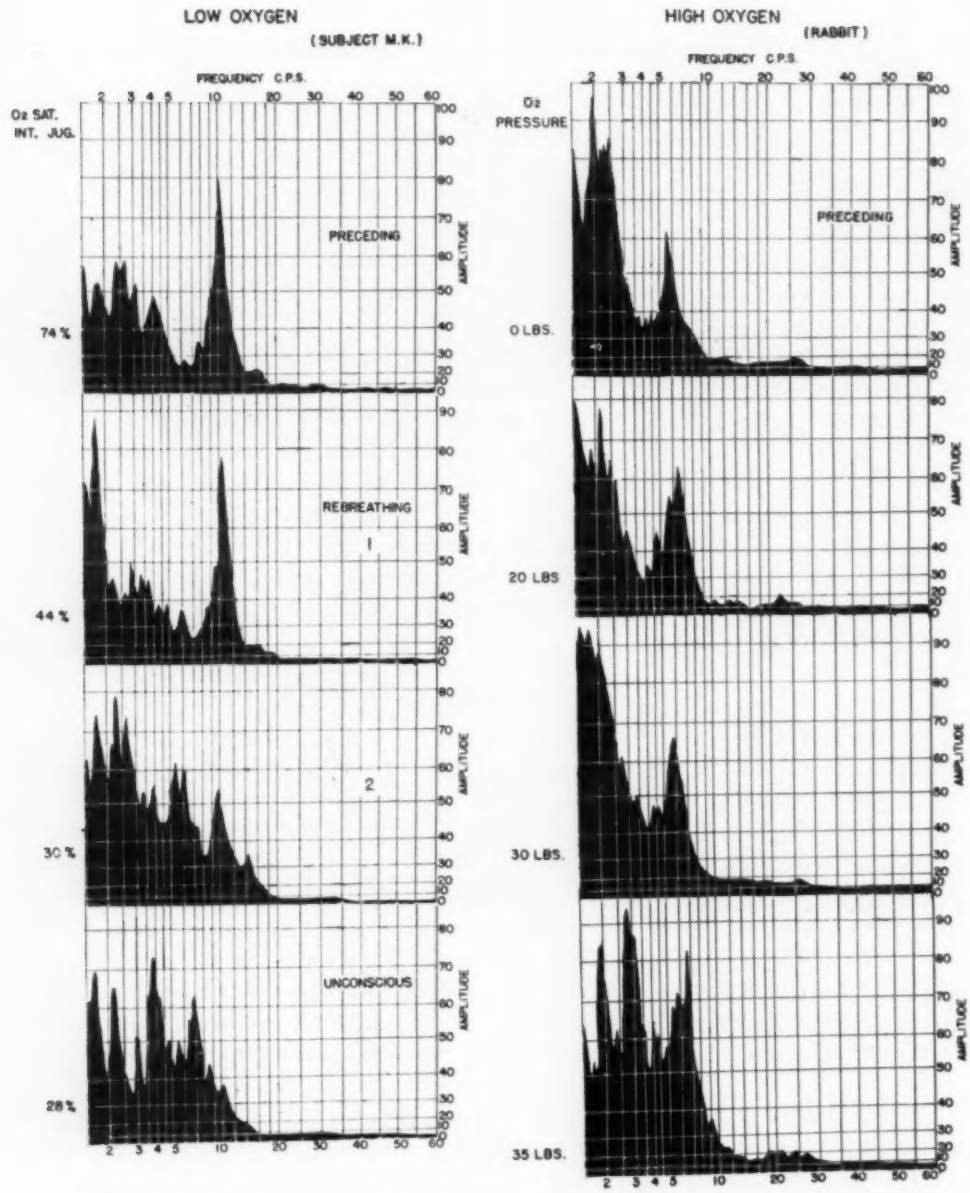

FIG. 5 (*Left*). Changes in the cortical frequency spectrum are equivocal or in the direction of slight slowing when the O_2 saturation in the internal jugular vein falls to 44 per cent. At 30 per cent, however, there is a marked shift to the slow side which becomes more pronounced when the O_2 level drops to 28 per cent saturated in the internal jugular. At or slightly below this level the total energy in the spectrum is decreased. The subject was unconscious during the 30-sec. period covered by the last spectrum.

FIG. 6 (*Right*). Changes are equivocal or in the direction of a slight speeding up until the O_2 pressure is raised to 35 lbs. of pure O_2. At this pressure the 6-per-sec. peak moves toward 7, a peak appears at 9 where there had been none before and a relative increase in energy occurs in the 18- to 30-per-sec. region. At this pressure or slightly above there is an increase in the total energy in the spectrum. It is obvious that this movement toward the fast side cannot continue indefinitely. With pressures of 45 lbs. the animal usually has a convulsion following which the spectrum shifts to the slow side. Unless the O_2 pressure is reduced, there is another shift to the fast side, another convulsion occurs and the final shift toward slow with the decrease in total energy which is associated with death.

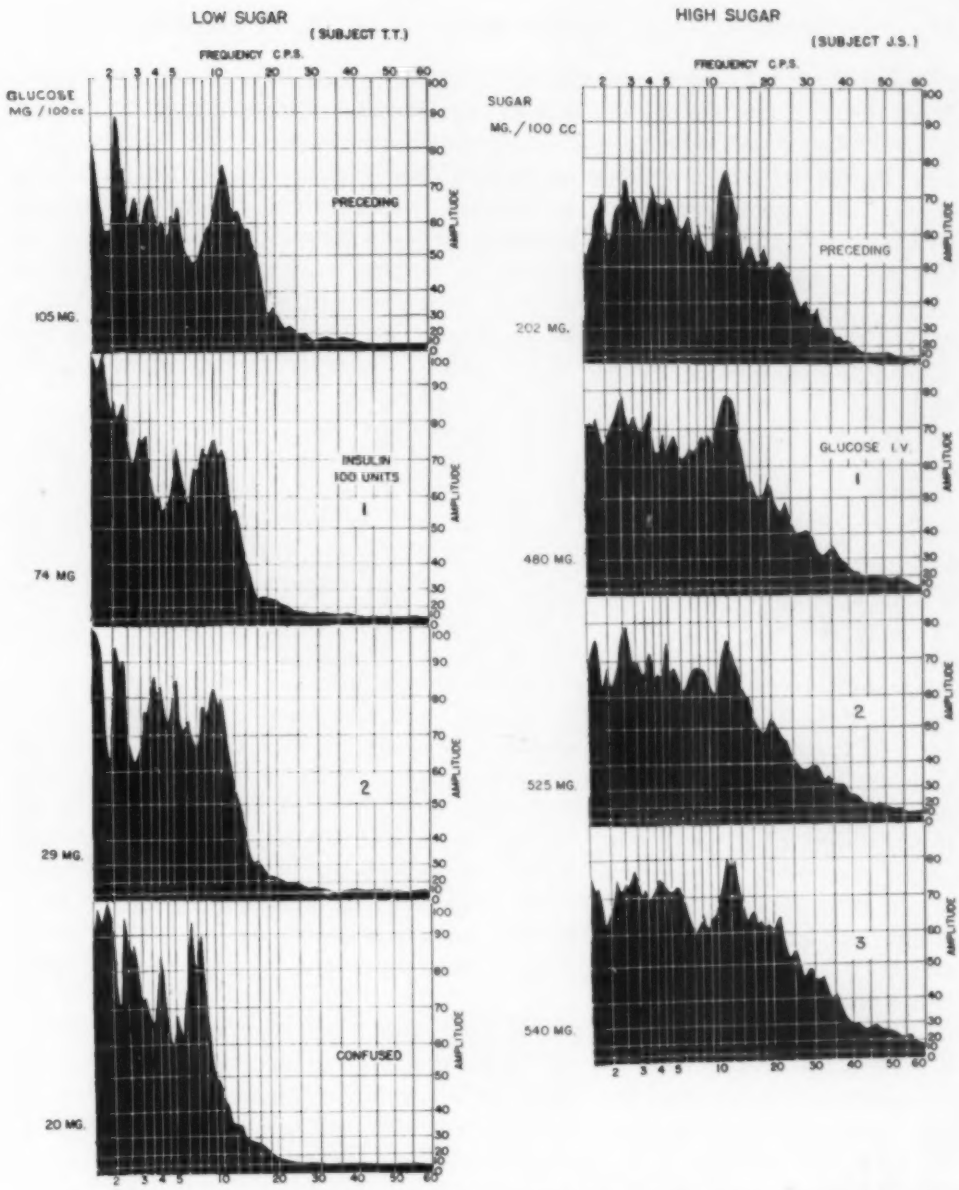

FIG. 7 (*Left*). With the fall from 105 to 74 mg. of sugar per 100 cc. of internal jugular blood there is evidence of an increase in energy in the slow frequencies and a decrease in energy in the 16-per-sec. region, but there is no definite shift in peaks until the sugar level falls below 29 mg. per 100 cc. The subject was confused during the 30-sec. period covered by the last spectum.

FIG. 8 (*Right*). Slight shift to the fast side of the spectrum can be detected when the sugar level is raised from 202 to 480 mg. per 100 cc. of internal jugular blood. A very evident shift in the form of a great increase in the energy in the frequencies above 15 per second occurs when the sugar level is changed from 525 to 540 mg. per 100 cc. This shift toward the fast side is associated with an increase in the total amount of energy in the spectrum.

twice the frequency at which the slow peak occurred may gain in amplitude. Such changes are usually evident in the unanalyzed record as a disappearance of 10-per-sec. waves and the appearance of many more 18- to 20-per-sec. waves, to cite a specific example. One cannot say what part of the energy at a given frequency is derived from relatively pure sign waves, which would appear as discrete and countable waves of that frequency in the unanalyzed record, and what part is derived from non-sinusoidal slower frequencies, the faster components of which are quite properly recorded as higher frequencies. In any case, it is permissible and profitable to group them together and to talk about the energy delivered at a given frequency as though it emanated from oscillators operating at that frequency.

In describing the changes occurring in the cortical frequency spectrum, reference has been made to an "apparent" increase in total energy, when there is an increase in the total area under the curve. An increase in area, such as occurs with low CO_2, does not necessarily indicate an increase in the amount of potential energy being used in the brain, but may indicate only an increase in the efficiency of the oscillators responsible for the electroencephalogram when they are operated at slower frequencies. This is a characteristic of all oscillators in which an inertia-like factor can control frequency. Rate of diffusion would be such a factor in an electro-chemical oscillator, such as is under consideration here. Likewise, a decrease in amplitude with an increase in frequency, such as occurs with high CO_2, does not necessarily indicate that less potential energy is being used, but may indicate only that the oscillators are less efficient at high frequencies. A shift toward the fast end of the spectrum, however, with an increase in the total area under the curve, as occurs with high O_2 pressure and with high blood sugar levels, strongly suggests an increase in the total potential energy being used. A decrease in the area under the curve, such as occurs with low O_2 pressure and with low blood sugar, suggests a decrease in the total potential energy being used.

Careful study of a consecutive series of spectra in which energy is gradually shifting reveals that a peak of energy at a given frequency tends to remain more or less constant while the slopes of the peak are pushed further and further in the direction of the force which is operating, for example, to the slow side with low CO_2. This suggests that the majority of oscillators at that frequency tend to be fairly stable, but that a certain number are more easily disturbed than others. If one considers the hypothetical case in which some oscillators having a frequency of, let us say, 10 per sec. are slowed to 9.5 and others which have a frequency of 10.5 per sec. are slowed to 10, the peak may remain in the same position but the sides of the peak may change. When, however, the force of the factor which is causing the shift becomes sufficiently strong, the entire peak shifts. Its movement appears to be limited, however, for as the main peak moves from, say, 10 to 9.5 per sec., its height rapidly diminishes. At the same time, the slower peaks gain in height as though they were absorbing the energy lost by the disappearing

faster peak. In some cases, the frequencies appear to gain more than the diminishing peak has lost, and at times a peak may decrease without much evidence of an increase in the height of a neighboring peak. These observations suggest that there are natural periods at which these oscillators operate most efficiently.

Summary and Conclusions

The responses of the electrical activity of the human cortex to alterations in normal blood constituents have been analyzed with the Grass frequency analyzer. Decrease in CO_2 content in the internal jugular blood is associated with a shift in energy distribution in the cortical frequency spectrum toward the slow side; an increase with a shift in energy distribution to the fast side; these effects become less marked with extremely high or extremely low CO_2 tensions. Oxygen and glucose, on the other hand, can be varied within wide limits with little change in the cortical frequency spectrum but when the O_2 saturation or glucose concentration in the internal jugular falls to a critically low level, there is a sudden shift of energy distribution to the slow side. With exceedingly high concentrations of glucose, the energy distribution in the cortical frequency spectrum shifts toward the fast side. Experiments on rabbits indicate that the effects of high O_2 tension are similar to the effects of high concentrations of glucose.

The results suggest that the electrical activity of the cortex is a manifestation of the activity of a great number of chemical oscillators having different natural periods. Though differing in their periods, these oscillators tend to respond similarly to any given factor. A factor which affects one frequency tends to affect all frequencies in the same direction, though not necessarily to the same degree.

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OCULAR ROTATION IN ANESTHESIA AND UNDER THE INFLUENCE OF SUPRANUCLEAR CENTERS

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I. INTRODUCTION

WHILE ocular movements in horizontal or vertical directions have been studied in much detail, very little is known about the influence of supranuclear centers on rotation of the eyeballs. In a study of ocular positions and movements in sleeping individuals, Pietrusky (1922) occasionally observed rotatory movements of the eyeballs. In a case of post-encephalitic hyperkinesis reported by Muenzer (1933), left-sided facial spasm was associated with rotation of both bulbs to the right; there was also slight paresis of the muscles supplied by the left V, VII, and XII nerves and of the left arm. The meagerness of our knowledge on this subject may be partly due to the fact that rotatory movements easily escape observation if one does not direct attention to this phenomenon, for instance by watching vessels in the sclera. Our study of this question was instigated by an accidental observation.

In the course of experiments on the brain-stem of cats, the appearance of a striking oblique position of the pupils was observed indicating an asymmetrical inward rotation of the eyeballs, *i.e.*, a rotation with the upper end of the vertical diameter of the corneas inward, around the axis through the anterior and posterior poles of the eyeballs. In an attempt to analyze this phenomenon, the question presented itself as to how far this rotation was due to the anesthetic (nembutal 35–40 mg. per kg., intraperitoneally). In fact, a similar rotation appeared under the influence of nembutal alone. It seemed, therefore, desirable first to study systematically the effects of anesthetics (experiments on 67 cats.)

METHOD

Before and during the course of anesthesia, the position of the pupil or of a linear scar, burnt into the cocainized cornea as closely as possible to the vertical diameter, was recorded by using a transparent celluloid protractor whose lower edge fitted into and was made movable along a deep groove in a horizontal metal bar fixed to the head-holder, parallel to the mouth.

For a study of the influence of supranuclear centers by stimulation and extirpation experiments, it was always necessary to burn scars into the corneas, since dilatation of the pupils often occurs, and since the pupils may be used as indicators of the ocular rotation only when they are slit-like or oval. To avoid the effect of the anesthetic as much as possible, these experiments were performed in superficial ether anesthesia.

II. RESULTS

The effects of anesthesia are summarized in Table 1.

Table 1. Effects of anesthetics.

Anesthetic	Number of animals	Average rotation		Range of rotation		Number of cases showing					
		Right eye	Left eye	Right eye	Left eye	Bilateral inward rotation	Bilateral outward rotation	Bilat. rot. in same direction	No rot. in one eye In. in other	No rot. in one eye Out. in other	No rotation
Ether	9	0.85° out	0.23° in	6½° out to 4½° in	3½° out to 4° in	3	3	2	0	2	3
Chloroform	4	0.56° in	0.81° in	5½° out to 8° in	4° out to 7° in	3	1	1	1	2	0
Gréhant's mixture	6	0.42° out	0.5° out	8½° out to 7° in	7½° out to 7½° in	0	1	3	2	0	0
Chloralose	7	7.07° in	13.14° in	2½° out to 30° in	2½° in to 34° in	5	0	1	1	0	0
Urethane	7	6.14° in	10.71° in	15° out to 20° in	4½° out to 26° in	5	1	1	0	0	0
Dial-urethane	6	12.00° in	8.00° out	1½° out to more than 25° in	17½° out to ½° in	1	1	3	1	0	0
Sodium barbital	6	8.58° in	9.42° in	22° out to 25° in	0° to 30° in	4	0	1	1	0	0
Sodium phenobarbital	6	12.67° in	7.67° in	0° to 18° in	5° out to 18½° in	4	0	1	1	0	0
Sodium pentobarbital	16	5.31° in	5.36° in	7½° out to 24° in	30° out to 35° in	12	2	3	0	0	0

The smallest range of rotation (8.5° outward to 8° inward) was noticed under the influence of ether, chloroform, or Gréhant's mixture (5 per cent chloroform in 50 per cent alcohol). A variety of effects could be observed: in- or outward rotation of one eye only or of both eyes, and also rotation of both eyes in the same direction ("homonymous rotation").

The effect of barbiturates (sodium barbital, sodium phenobarbital, sodium pentobarbital, dial-urethane,*) and also of chloralose and urethane was much more marked, the range of rotation reaching from 35° inward to 30° outward.

In the majority of the cases of this second group, the eyes were inwardly rotated (bilateral inward rotation in 31 of 49 observations; inward rotation of only one eye in 4 cases). Much more rarely, bilateral outward rotation appeared (4 instances of bilateral outward rotation of relatively slight degree), or rotation of both eyeballs in the same direction (10 observations). The tendency for homonymous rotation was manifested particularly in dial-urethane anesthesia (3 of 6 cases). In the cases with heteronymous as well as in those of homonymous rotation, it was usually more marked in one eye than in the other.

The extent of the rotation was subject to fluctuation during the anesthesia, *e.g.*, in one stage the right eye might be markedly inwardly rotated and the left only slightly, while at a later stage the opposite might be observed. Often, however, the eyeballs retained the same degree of rotation for several hours. Involuntary horizontal and more rarely vertical movements of the eyeballs (undulation or nystagmoid movements) appeared in some experiments, but the rotation was as a rule independent of these motor phenomena.

Thus, particularly those anesthetics that act mainly on the brain-stem, such as the barbiturates, produce a marked ocular rotation, while so-called cortex anesthetics (Pick and Molitor, 1929), such as ether, chloroform, or chloroform-alcohol show such an effect to a slight degree only. A sharp division between cortex- and brain-stem anesthetics seems, however, impossible since a so-called cortex anesthetic (chloralose) may produce a marked bilateral inward rotation.

For further analysis of these phenomena, it seemed of interest to study the influence of various parts of the central nervous system upon the position of the eyeballs with special reference to rotation around the antero-posterior axis of the eye (experiments on 25 cats).

Stimulation of the cortical centers for ocular movements in the frontal or occipital lobe produced, besides conjugate deviation of the eyeballs to the opposite side and less frequently vertical deviation, a slight rotation in the majority of the experiments (Table 2). The most frequent reaction was a rotation of the opposite eye towards the stimulated hemisphere (in regard to the upper end of the vertical diameter of the cornea, average less than

* The dial was kindly supplied by Ciba Pharmaceutical Products Inc.

5°, maximum 15°). The reaction of the homolateral eye was more variable. The inward rotation of the opposite eye might be accompanied by a slighter inward rotation of the homolateral eye; or the homolateral eye might rotate outwardly so that both eyes rotated towards the stimulated cortex; or a reaction of the ipsilateral eye might be absent. In a few instances, rotation was observed in the homolateral eye only (outward rotation), or this reaction was accompanied by a weaker outward rotation of the opposite eye. Extirpation of the frontal or occipital oculogyric centers or combined elimination of these areas resulted only in a slight and temporary inward rotation reaching not more than a few degrees. Thus, a definite tonic effect of the cortex upon the position of the eyes in regard to rotation could not be found.

Table 2. Stimulation of cortical oculogyric centers.

Rotation of contralateral eyeball	Rotation of ipsilateral eyeball	Number of observations
no rotation	no rotation	12
inward (to stimulated cortex)	inward (to non-stimulated cortex)	10
inward (to stimulated cortex)	outward (to stimulated cortex)	7 (22)
inward (to stimulated cortex)	no rotation	5
no rotation	outward	2
outward	outward	4
		40

After combined extirpation of the prosencephalon and diencephalon by a section in front of the midbrain and after discontinuation of the ether anesthesia, inward as well as outward rotation of one or both eyeballs could be observed (5 times both eyes inward, twice both eyes outward, once homonymous rotation, once no rotation). Only in 3 out of 9 experiments was the vertical diameter of the cornea rotated by more than 10° from the vertical position; the maximum rotation observed was 20° inward rotation on the left and 2.5° inward rotation in the right eye. In this case the anatomic examination revealed that the section traversed the brain-stem dorsally in front of the superior colliculi, ventrally just in front of the chiasma, while in the other cases the ventral end of the section was just in front of the cerebral peduncles or through the tuber cinereum.

Such high degrees of rotation as were obtained under the influence of such anesthetics as the barbiturates and chloralose were, however, not reached in these extirpation experiments. This may be partly due to the fact that the tone of the mesencephalic ocular nuclei may become impaired after section in front of the midbrain as a consequence of shock, as indicated by a more or less pronounced dilatation of the pupils. Furthermore, the possibility has to be borne in mind that the rotation in anesthesia may be produced

not only by elimination of the action of higher centers upon the mesencephalic ocular nuclei but also by a direct action of the anesthetics upon parts of the brain-stem below the diencephalon. For dial, an action upon the oblongata is suggested by such symptoms as salivation, vomiting, and occasional respiratory failure (Fulton, Liddell, and Rioch, 1930).

In our observations, an action upon the rhombencephalon, particularly the vestibular nuclei, is suggested by cases of anesthesia with marked homonymous ocular rotation, which is usually of unequal degree in both eyes. This homonymous rotation may reach higher degrees (up to 25° in unoperated animals) than that observed in the above-mentioned experiments with extirpation of prosencephalon and diencephalon. Now it is known that a marked homonymous rotation to the side of operation appears after unilateral labyrinthectomy (Rademaker, 1935). Godlowski (1938) observed homonymous rotation to the stimulated side on stimulation of the most cranial parts of the posterior longitudinal fasciculus, leaving it undecided whether this effect was due to ascending or descending fibers.

Our experiments showed that the rotation to the operated side following unilateral labyrinthectomy is more developed in the opposite eye (average 20°) than in the homolateral eye (average 7°). A similar type of rotation may be obtained by a unilateral lesion of the vestibular nuclei (incision on the inner side of the restiform body). The rotation may still be observed after the nystagmus following the labyrinthectomy subsides, and also without accompanying nystagmus on a lesion of the vestibular nuclei in anesthetized animals. Thus, the fact that the homonymous rotation in anesthesia may appear without nystagmus does not speak against the possibility that the effect of the anesthetics may reach in these cases the rhombencephalic vestibular nuclei disturbing the balance between the nuclei of the two sides.

In order to test the possibility of a direct action upon the centers behind the thalamus, transverse sections immediately in front of the superior colliculi and the cerebral peduncles were performed in ether anesthesia, which was discontinued after this operation. Some time later, after the position of the eyeballs in regard to rotation had become fairly constant, a brain-stem anesthetic (usually dial-urethane) was injected intraperitoneally. While in some experiments the change in degree of ocular rotation was only slight and transitory, in others it was marked (maximum in one case was 11° outward, in another case 17.5° inward) and lasted for hours. This corroborates the assumption of a direct action of the anesthesia upon parts of the brain-stem behind the diencephalon.

An attempt was made to ascertain whether anesthetics may still produce ocular rotation if the mesencephalon is separated from the supranuclear rhombencephalic centers. After ocular rotation had developed in otherwise normal animals under the influence of barbiturates (nembutal, dial-urethane), a transverse section through the most cranial part of the pons was performed. Such a section diminished the rotation produced by the anesthetic but failed to abolish it, while the contraction of the pupils indicated

that the mesencephalic eye muscle nuclei were still in a good condition (*e.g.*, inward rotation in nembutal anesthesia on the right eye 12–15° before, 10–13° after the section; on the left eye 8–12° before, 5–7° after the section; pupil diameter on the right side 3 mm. and on the left side 4 mm. after the section). Similar observations were made in mid-brain animals in favorable cases (*e.g.*, bilateral outward rotation produced by dial-urethane was diminished but not abolished by transverse section behind the posterior colliculi sparing the basilar artery). Thus on one hand cases of homonymous rotation suggest that the action of the barbiturates may reach as far down as the vestibular nuclei; on the other hand it could be shown that an action upon rhombencephalic supranuclear centers is dispensable, and that a direct action upon the mesencephalon also exists, particularly in the production of heteronymous rotation.

Ligature of the carotid arteries, which procedure, according to de Kleyn and Versteegh (1922), keeps toxins circulating in the blood away from the eye muscles, did not prevent the appearance of ocular rotation under the influence of anesthetics.

Summary

1. Under the influence of anesthetics, particularly of the so-called brain-stem anesthetics, various types of ocular rotation (heteronymous and homonymous rotation) occur.

2. Stimulation of the cortical oculogyric centers may produce, besides conjugate deviation in a horizontal or vertical direction, a slight rotation (most frequent reaction: rotation of the opposite eye towards the side of stimulation). Tonic effects of the cortex upon the position of the eyeballs in regard to rotation could not be found. Elimination of the cortex plus subcortical ganglia in front of the midbrain produces moderate degrees of rotation, but not such high degrees of rotation as are observed under the influence of anesthetics.

3. After unilateral labyrinthectomy, rotation to the side of the operation is more marked on the opposite side than on the homolateral eye; this rotation may outlast the spontaneous nystagmus. A similar homonymous rotation may be produced by a unilateral lesion of the vestibular nuclei, suggesting that disturbances of the equilibrium between the vestibular nuclei of the two sides may play a part in the genesis of homonymous rotation observed in barbiturate anesthesia.

4. Ocular rotation is still produced by brain-stem anesthetics such as dial-urethane in mid-brain animals. It is inferred that the ocular rotation produced by anesthetics is only partly due to depression of prosencephalic and diencephalic activity and partly to direct action upon the lower centers.

5. Separation of the mesencephalon from the rhombencephalon in normal as well as in mid-brain animals diminishes the rotation produced by barbiturates, but does not abolish this rotatory effect; this indicates a direct action of the anesthetic upon the midbrain, besides the action upon rhombencephalic supranuclear centers.

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EFFECT OF VARIOUS CORTICAL LESIONS ON DEVELOPMENT OF PLACING AND HOPPING REACTIONS IN RATS

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REMOVAL of the sensorimotor areas of the cerebral cortex from a variety of mammals causes permanent deficiencies in placing and hopping responses directly proportional to the degree of cortical control characteristic of the particular species (Bard, 1933; Brooks, 1933; Woolsey, 1933; Brooks and Woolsey, 1936; Woolsey and Bard, 1936). Even in rats the control of placing reactions is so strictly localized in the sensorimotor cortex that no observable recovery of these functions occurs following ablation of that area. These placing and hopping responses, first described by Rademaker (1931), are not present in most mammals at birth. Several days are required for their full development. Yueh Tang (1936) has described the time and order of their appearance in rats and has also endeavored to correlate development of responses with phases of cerebral maturation.

In higher mammals certain changes resulting from cortical injury gradually disappear and deficiencies which are obvious immediately following cortical ablations become less apparent after several weeks (Hines, 1937). It is claimed by Kennard (1936, 1938) that a much greater degree of readjustment or recovery occurs in monkeys if lesions are made in infancy. No detectable improvement in hopping and placing responses of adult rats was observable (Brooks, 1933) after these reactions had been rendered deficient (hopping) or abolished (tactile placing) by complete ablation of sensorimotor cortex. Hopping responses which are elicited by adduction, abduction or retroflexion of a leg are presumably initiated by proprioceptive stimuli. In adults, after removal of the sensorimotor areas hopping still occurs, but a greater degree of displacement is required for initiation of the reaction, *i.e.*, the response is rendered deficient, but not abolished. Placing reactions which occur when the vibrissae or the hairs of a foot or toe come in contact with an object are the result of tactile stimulation. These tactile, or contact placing responses are permanently abolished in the adult by ablation of the sensorimotor areas of the cortex. The question arises whether or not these abnormalities in response would result if the sensorimotor areas were removed from newborn rats before development of placing and hopping reactions. Possibly under these conditions other regions of the maturing cortex, or subcortical structures, would be capable of assuming a function normally dependent on the sensorimotor area.

METHODS

Eleven litters containing a total of 55 rats were used in this study. Preliminary operations were performed before development of the hopping and placing responses, *i.e.*, from the 1st to the 5th day after birth. The young animals were anesthetized with ether and various portions of the cerebral cortex were removed by means of a capillary pipette and gentle suction. These animals were observed daily from time of operation to time of autopsy and were found to be so slightly affected, even by lesions as extensive as hemidecortication or bilateral removal of the frontal third of the neocortex, that they had little difficulty competing with litter mates for food. Once each week during the month following operation the rats were tested for evidence of hopping and placing responses. The tests used in examining these animals were similar to those described by Brooks (1933). Two months after operation one of us, not knowing the site of the cortical lesions, made a detailed examination of each animal. On the basis of the type of deficiency observed predictions were made concerning the locations of the lesions. At this time either the sensorimotor or various other areas were removed from the intact hemispheres of ten of these animals which had been subjected previously to a unilateral ablation. We were able, in this way, to compare in the same animal effects of operations performed before and after appearance of placing and hopping reactions.

Two to 6 months later all animals were again tested. Following this final examination, the cortices of 19 of the rats were stimulated electrically. The extent of the lesions in the sensorimotor area and the electrical excitability of various cortical remnants were thus ascertained. The stimulator employed delivered a 60-cycle sinusoidal wave. Voltage and current strengths were recorded simultaneously during stimulation. A unipolar electrode was used for exploration of the cortex, the indifferent electrode being placed on the abdomen. The remaining rats were autopsied and their brains studied.

RESULTS

Our study of the development of placing and hopping responses of young rats revealed that although there is considerable variation in speed of maturation, reactions develop in an orderly sequence within ten days to two weeks after birth. The conclusions of Yueh Tang (1935) concerning the order and time of appearance of placing and hopping responses were thus confirmed. This development is apparently little, if at all, retarded by removal of electrically inexcitable areas of the cortex. Likewise, removal of a portion of sensorimotor cortex does not retard the appearance of leg reactions controlled by remaining portions of the area. Ablation of one hemisphere does not prevent normal development of these responses in limbs contralateral to the intact hemisphere.

Six animals which had undergone complete ablation of the sensorimotor area of one hemisphere, between 1 and 5 days after birth, failed to develop normal hopping and placing reactions in limbs contralateral to the lesion. Bilateral removal of electrically excitable cortex (one animal) shortly after birth resulted in maximal bilateral deficiencies; this animal never developed tactile reactions and hopping responses which did appear were as deficient as those of decorticate adults. Lesions involving not only the sensorimotor areas, but, in addition, other portions of the cortex, failed to cause detectably greater degrees of deficiency. The deficiencies of 4 hemidecorticate rats were indistinguishable from those of animals whose lesions included only the sensorimotor cortex of one hemisphere.

Deficiencies resulting from removal of the entire sensorimotor area during infancy appeared to be as great as those following removal of comparable

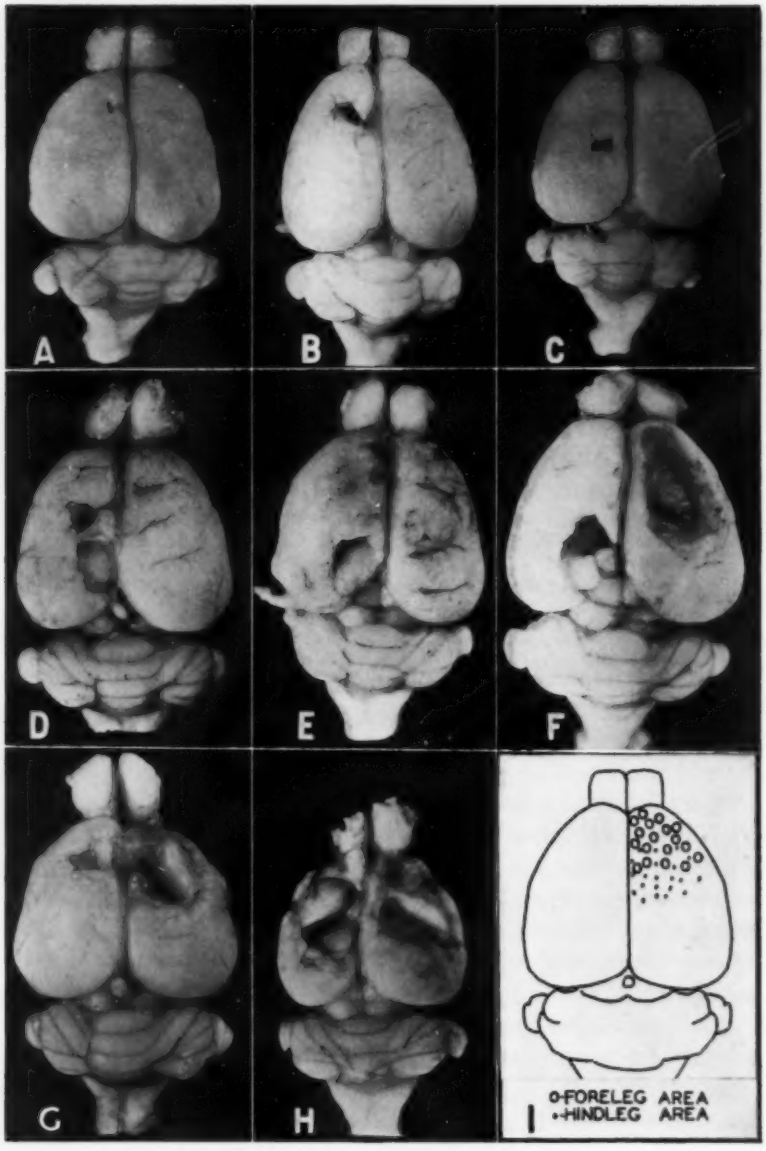

FIG. 1. A. A small cortical lesion made twelve days after birth which caused permanent, but barely detectable, deficiencies in reactions of contralateral foreleg.

B. A lesion which produced easily detectable deficiencies in the contralateral foreleg, but no apparent deficiencies in the reactions of contralateral hindleg. Lesion made five days after birth.

C. The reactions of both right fore and hindleg were rendered slightly deficient by this small lesion. The hindleg responses were affected to a greater degree than were those of the foreleg. No recovery was observed although the lesion was made two day after birth.

D. A lesion in the occipital region of the cortex which included a considerable portion

areas of adult rats. Five animals which had undergone unilateral removal of the sensorimotor area between days 1 and 5 were operated upon a second time, 15 weeks later. In this way we were able to compare, in the same animal, the permanent effects of ablation of the area shortly after birth and after maturation of all responses. No differences in deficiencies on the two sides could be detected in these preparations 2 months after the second operation (Fig. 1H).

In 36 young rats lesions were made which involved only portions of the electrically excitable areas of the cortex. These lesions varied in size and location. In 1 animal the lesion caused no detectable deficiency and in 3 any abnormalities of reaction, if present, were very slight after 5 months. At autopsy, minute lesions were found in the sensorimotor cortex. Stimulation of the cortex, moreover, showed that only a small fraction of the electrically excitable area had been removed.

Three mature rats which had been operated on during infancy showed impairment of the responses of the contralateral foreleg, but the hindleg responses were apparently normal. At autopsy, it was found that the cortical lesions were located within the foreleg area of the sensorimotor cortex (Fig. 1A and B). A few of these animals showed normal foreleg activity, but deficiency followed a small lesion confined to the hindleg area of the sensorimotor cortex. In 3 others a large occipital lesion had involved a portion of the hindleg area (Fig. 1D). Stimulation of the anterior margins of these lesions caused hindleg movements, but it was obvious that a considerable portion of the hindleg cortex had been ablated.

Twenty-eight rats with small unilateral cortical lesions showed a degree of deficiency in reactions of both opposite legs. When these rats were compared with hemidecorticate rats, or with others having complete removal of unilateral sensorimotor area, it appeared that the legs were not maximally

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of the hindleg area. Permanent deficiencies in the reactions of the contralateral hindleg resulted. Lesion made five days after birth.

E. A lesion in the occipital cortex which caused no detectable deficiencies in hopping and placing responses of the contralateral hindleg.

F. The lesion in the left cortex (made five days after birth) resulted in deficiencies which were confined to the contralateral hindleg. The lesion in the right hemisphere, made 54 days later, caused maximal deficiencies in the left legs. Right foreleg movements were elicited by electrical stimulation of that portion of the left hemisphere anterior to the lesion. No leg movements could be obtained by stimulating other portions of the cortex of either hemisphere.

G. The lesion in the left hemisphere (made five days after birth) rendered the right foreleg clearly deficient. The reactions of the right hindleg were affected to a lesser degree. Ablation of the sensorimotor cortex of the right hemisphere when the animal was six weeks old rendered the left legs more deficient than were the right. Stimulation of the cortex of this brain revealed that remnants of foreleg and hindleg areas remained in the left hemisphere.

H. The lesion in the left hemisphere was made two days after birth; that in the right hemisphere two months later. The deficiencies in the placing and hopping responses resulting from the two lesions were identical.

I. Electrically excitable areas as determined by stimulation of the right hemispheres of eleven rats.

deficient. To test this the sensorimotor cortex of the intact hemisphere was ablated. The legs contralateral to the new large lesion were more deficient than those contralateral to the smaller and older lesion. At the termination of these experiments exploration of the cortex containing the smaller lesion revealed the presence of a little tissue of both foreleg and hindleg areas (Fig. 1G). It was concluded that these areas were of some functional importance and were responsible for differences in the degree of deficiencies of the two sides.

In 5 rats, lesions made in infancy in the occipital cortex or other non-excitable areas did not result in deficiencies of response (Fig. 1E) at any time during survival periods of 6 months. Stimulation of the cortex of these animals showed a normal presence and distribution of electrically excitable areas. Figure 1-I is a composite diagram of the points from which electrical stimulation elicited foreleg and hindleg movements in eleven rats. These experiments show that removal of sensorimotor cortex from young rats before development of placing and hopping responses does effect the reactions permanently. No other areas were able to assume control of these responses. Even small lesions within these areas result in detectable permanent deficiencies.

On the basis of the deficiencies of each of these 55 animals, the locations of lesions were predicted. At autopsy it was found that only two serious errors in prediction had been made. In 9 additional cases the prediction was partially incorrect. That is, in these animals which showed some deficiencies in both fore and hindleg response, mistakes were made when we attempted to judge whether the larger part of the lesion was in the fore or hindleg area.

Examination of the pyramidal tracts above the decussation, revealed a few points of interest. Those animals in which a small lesion had been made in the foreleg or hindleg area showed a reduction in size of the bundle ipsilateral to the lesion. In those animals in which an occipital lesion had encroached upon the hindleg area, there was a smaller pyramid on the ipsilateral side. Occipital lesions which did not impinge upon sensorimotor cortex did not detectably modify the pyramid. In those animals in which deficiences were not maximal pyramidal fibers were sufficiently numerous to be detected macroscopically. The reduction in size was roughly proportional to the magnitude of the sensorimotor lesion. Completely hemidecorticate animals, and those in which the frontal portions of the cortex (sensorimotor area) had been removed, possessed no macroscopically visible pyramid ipsilateral to that hemisphere. Histological study of some of these brains, however, revealed a few fibers which might have been incorporated in that tract.

Discussion

The placing and hopping reactions are maximally developed in adult rats but some such as the hopping response to adduction of a leg, can be rendered

only slightly abnormal by removal of the cortex (Brooks, 1933). No difficulty was encountered in determining the presence or absence of contact placing and the other responses which were absent after complete removal of the sensorimotor area, but the degree of deficiency of hopping reactions was harder to judge. Small rats were more active than adults and some difficulty was encountered in studying their responses. Consequently, a great deal of variation was seen, but it may be explained as due either to fluctuations in emotional state of the animal, *i.e.*, the degree of excitement, or to the manner in which the animal was held, *i.e.*, in painful or uncomfortable positions. Nevertheless, even in young animals, it was possible to determine slight deficiencies.

It can be concluded that a small lesion within the foreleg or hindleg cortical area produces detectable abnormalities of a permanent nature, even when the operation is performed on the first postnatal day. The entire foreleg or hindleg area must be ablated, however, before the state of maximal deficiency in these reactions is attained. The maximal deficiency is that state which results from complete decortication or complete removal of the frontal half of the neocortex.

Incomplete removal of the sensorimotor area was followed by lesser degree of permanent deficiency than was complete ablation of the area. The degree of deficiency was estimated in this way. It was seen that the responses of legs contralateral to injured cortex were deficient when compared with the responses of legs contralateral to the intact hemisphere, but the reactions of the affected legs of some rats were more easily elicited, more prompt and more uniform than those of other rats. This difference might be explained on the basis of individual variations, but a second method of comparison proved this to be an inadequate explanation. In several of those rats which clearly showed contralateral deficiencies after a first operation, but whose responses were definitely superior to those of animals with larger lesions, we ablated the entire sensorimotor cortex of the intact hemisphere. The legs contralateral to this lesion became permanently more deficient than were the legs opposite the smaller original lesion. This second method of comparing deficiencies gave even stronger proof than the different degrees of deficiency resulted from unequal subtotal lesions. When the original lesion included the entire sensorimotor cortex of one hemisphere removal, from the mature rat, of a comparable area of the other hemisphere resulted in deficiencies of the postural reactions which were equal on the two sides.

Brooks (1933) found that removal of the sensorimotor areas from the cortex of the adult rat produced permanent deficiencies in the postural reactions of the legs contralateral to the lesion. The experiments reported at the present time justify the statement that if any recovery occurs after partial or complete ablation of sensorimotor areas it is never sufficient to restore the placing and hopping responses to normal, even when the lesions are small and are made within a few hours after birth. It is more difficult to determine whether or not partial recovery of these placing and hopping responses takes

place after they have been rendered deficient by ablations involving only a part of the sensorimotor cortex. The infant rat has none of these postural reactions and, consequently, only the ultimate state of deficiency can be ascertained. In the adult rat and in young rats, which have developed the placing and hopping responses, all reactions are somewhat depressed immediately following ablation of a portion of the cortex. The placing and hopping deficiencies are more noticeable then than they are a few hours later. It is conceivable that this temporarily greater impairment of response is due entirely to after effects of the anesthetic, mild surgical shock or to such temporary conditions as irritation, edema, or an impairment of circulation in the subcortical structures. In the original studies by Brooks (1933) it was concluded that improvement in the responses is probably due to general recovery of normal activity rather than to an assumption of a new function by the remaining tissues. The temporary depression observed in the reactions of limbs opposite intact cortex was likewise interpreted to be an indication of a general depression of all activity rather than an indication of an ipsilateral effect of the ablation. This study of young animals has furnished no evidence which would justify a modification of this conclusion.

It also seems reasonable to assume that another reason why the animals appear to be more deficient immediately after operation than they are a few hours later is that readjustments of a complex nature occur. The animals probably learn to avoid movements and reactions which they are unable to execute properly. They use other methods and mechanisms for attaining the same end. This might be called readjustment rather than recovery, if by recovery is meant the reappearance of a reaction which involves the use of the same muscle groups, and if the term recovery implies that another portion of the brain has assumed a function normally executed by the sensorimotor cortex.

The results of this work justify two general conclusions. First, the cortical control of the placing and hopping reactions is strictly localized to the sensorimotor cortex. Even within this general area there is at least some subdivision of function. These localized areas are of paramount importance to any reaction pattern involving postural adjustments. Secondly, no subcortical structure and no other portion of the cortex can assume, even during infancy, this control of the placing and hopping which is normally localized in a particular portion of the sensorimotor area.

Summary

Complete removal of that area of the cortex which in rats 1 to 5 days old corresponds to the sensorimotor area of the adult results in a permanent deficiency of the placing and hopping responses. This deficiency is indistinguishable from that caused by a similar operation on the other hemisphere of the same rat after it had reached maturity.

Small lesions confined to the foreleg or hindleg areas produce permanently detectable deficiencies in these postural reactions. It is possible to

determine the position and extent of the lesion by a study of the deficiencies in the placing and hopping reactions. The deficiencies following incomplete ablation of the sensorimotor area are not as great as those resulting from complete removal of this portion of the cortex.

There is no detectable improvement in the placing and hopping reactions after they have been rendered deficient by removal of the sensorimotor cortex. Even when lesions are made in the sensorimotor cortex immediately after birth, the remaining cortical and subcortical tissues are unable to assume, as they mature, the function normally executed by that portion of the sensorimotor cortex which has been ablated.

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ACTIVITY OF ISOCORTEX AND HIPPOCAMPUS: ELECTRICAL STUDIES WITH MICRO-ELECTRODES

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INTRODUCTION

IT IS clear that methods are needed for dissecting, physiologically, the electrical activity of the nerve centers, because it is only by limiting to one or a few the number of units which are contributing appreciably to the activity recorded that one may make clear-cut deductions about the processes occurring in the units. This has been attempted in the present experiments by investigating the electrical activity of structurally favorable regions of the brain by means of very small recording electrodes such as have been used in a few previous investigations of nerve and muscle (Gelfan and Bishop, 1932; Hogg, Goss, and Cole, 1934; Barron and Matthews, 1935; Jasper, 1936; Buchthal, 1937; Buchthal and Lindhard, 1937; Dusser de Barenne and McCulloch, 1938). In some of the experiments the choice of a type of anesthesia which results in relatively simple electrical patterns has aided the analysis.

In the interpretation of the present results and the data of the literature, validity is assumed for the following propositions which follow from the potential theory (cf. Pierce, 1902).*

(a) The potential at a point in a conducting medium due to the existence of activity in portions of a cell which is at a distant part of the medium is small (either approaches or actually is zero as the distance of the point from the cell increases). Therefore an electrode remote from a nerve center may be considered as indifferent with regard to electrical activity in that center.

(b) There is no external electrical field set up and therefore no current flows in the medium about a cell so long as every part of the surface of the cell is at the same potential. Even if the potential difference between the

* Serious deviations might occur if barriers or laminae of high impedance were interposed in various positions within the parts of the nervous system under consideration; in our experiments the brain has proved sufficiently homogeneous to preclude appreciable discrepancies. These propositions, of course, represent no original contribution for, as Lorente de Nó (1939) has recently pointed out in an excellent statement of the subject, potential theory was applied to the study of currents of action and injury by Helmholtz as long ago as 1852. We have found the data presented by Craib (1927, 1928, 1930) and by Bishop and Gilson (1929) most informative; the monograph of Wilson, MacLeod and Barker (1933) has also been useful. Our interpretations have been in terms of the membrane theory, although, as Helmholtz indicated, examination of the electric field in the medium about cells does not uniquely define the mode of origin of the loci of the sources of potential difference. Some recent data suggest the possibility that some of the voltage differences recordable from organisms may be due to concentration gradients arising from the diffusion of metabolites in the milieu surrounding cells (cf. Burr and Northrup, 1939; Teorell, 1935).

outside and the inside of the cell should change greatly, no external field would be set up if the change took place uniformly and simultaneously over the entire surface.

(c) An external electric field is set up when a potential difference occurs between any two regions on the surface of the cell. Current flows from the regions of high potential to those of low through the surrounding medium and returns within the cell. Points in the medium close to a region of the cell surface which is at a relatively low potential are negative to the distant point *p*; points close to a region which is at relatively high potential are positive to the point *p*.

(d) The difference in potential between any point *n* near the cell and a distant point (which is at approximately average or zero potential) due to the condition described in (c) decreases very rapidly as the distance of *n* from the cell increases. The density of current becomes rapidly greater as an active portion of a cell is approached, because of the decreasing volume through which the action current may flow; consequently, assuming approximately constant specific resistance of the medium, the IR drop becomes rapidly steeper as the active region (or an adjacent inactive region) is approached.

The basis for one valuable use of micro-electrodes is revealed in these propositions, and particularly in (d). It follows that, in records of the potential differences occurring between an indifferent electrode and a micro-electrode placed in a region where active units are randomly distributed, the potential changes contributed by units which have an active region (or an adjacent inactive region) very close to the micro-electrode will be very much greater than those contributed by units farther away. Therefore as an approximation the record consists largely of potential changes due to units close to the micro-electrode, and the volume of tissue contributing effectively to the record is greatly reduced. The data presented below verify the two corollaries, that (1) micro-electrodes should record in a localized way under certain circumstances and (2) large potential differences should be recorded by micro-electrodes placed in certain positions in active tissue. A second use of very small electrodes is the determination of the spatial distribution of potential in a mass of tissue without the infliction of much damage. Our results demonstrate that particularly interesting deductions may be made from such data obtained in regions of the nervous system where the spatial arrangement of cells is particularly simple, as it is in the hippocampus.

Preliminary reports of some of our findings have been presented previously (Forbes, Renshaw and Rempel, 1937; Renshaw, Forbes, and Drury, 1938; Renshaw and Forbes, 1938).

METHODS

Micro-electrodes devised for recording potential changes of low (*i.e.*, physiological) voltage in tissues should possess, as far as possible, certain characteristics. When the potential changes investigated are small, as is often the case in the nervous system, it is necessary to obtain as large a signal-to-noise ratio as possible. Since this ratio is an inverse function

of the resistance of the input circuit of the amplifier, low electrode resistance increases the resolution of the recording system.

Unlimited resolution of small electrical variations is not possible in spite of the tremendous voltage and current amplifications made available by the vacuum tube (Schottky, 1918; Johnson, 1928; Nyquist, 1928; Llewellyn, 1930). For a properly designed amplifier used with an input circuit of high resistance, the thermal noise originating in the resistance is the factor which mainly limits the value of the ratio, signal-to-noise, for small signals. The thermal noise-level—that is, the magnitude of the spontaneous, fluctuating potential changes measured across the ends of any resistance—depends only on three variables, the absolute temperature of the conductor, its resistance, and the frequency-band to which the recording instrument is sensitive, and varies with the square root of each of these quantities. For the study of physiological transients the frequency-band should extend from approximately zero to about 10,000 cycles per sec., and the temperature is not amenable to reduction. Thus the resistance of the input circuit becomes the only factor which may be altered to reduce the noise-level of the recording apparatus. Using the amplifier and cathode ray tube, noise-levels have been determined for various values of the total resistance of the input circuit as shown in Table 1; the values seem large because they

Table 1

Total resistance (MΩ)	Noise-level (width of baseline in μV)
0.1	15
1.0	45
10.	150

Table 2

Electrode resistance (MΩ)	Grid resistance (MΩ)			
	0.5	1.0	10.	100
0.01	9.90	9.95	10.0	10.0
0.1	2.88	3.02	3.15	3.16
1.0	0.577	0.707	0.953	0.995
10.	0.0686	0.0954	0.224	0.301

represent more nearly peak-to-peak than the usual root-mean-square values. Since the grid resistor of the amplifier is in parallel with the electrodes, reduction of the grid leak reduces the noise-level, but it also reduces the size of the signal as recorded. Table 2, giving relative values (calculated for small signals and considering only thermal noise in the input circuit) for the ratio, signal-to-noise, as electrode resistance and grid resistance are varied, demonstrates that when high-resistance electrodes are used the degree of resolution obtainable varies inversely with the electrode resistance and directly with the grid resistance. The decreased resolution accompanying the use of high-resistance micro-electrodes is compensated for by the surprisingly large potentials often recorded with them.

Other desirable characteristics in micro-electrodes are electrical stability, non-polarizability and small size; in order to minimize injury to the tissue into which the micro-electrodes are inserted, their inert walls should be as thin as is compatible with proper strength and insulation and their taper back from the small tip should not be greater than considerations of rigidity and electrical resistance demand.

The available micro-electrodes fall into two groups. In one a metal surface of small area is in contact with the tissue (cf. Taylor and Whitaker, 1927); in the other type the lead to the tissue is made by a salt bridge which is microscopic in area at the tip, and the metal interface of the half-cell may be large (cf. Ettisch and Peterfi, 1925; Gelfan, 1927). A type of micro-electrode satisfactory for the present purposes has proved to be of the second group. It is a silver-silver chloride Ringer-agar half-cell in which a chlorided silver

wire dips into a glass micropipet filled with a gel of agar in Ringer's solution (Fig. 1).* The tip diameters have varied upward from 15 μ and the wall thicknesses have been but a small fraction of the whole. Since tissue fluid and Ringer's solution have approximately the same ionic composition, diffusion potentials at the pipet tips must be small. Further there is no danger of diffusion from the pipet of ions which might change or affect the excitability of tissues; this is a danger when the salt bridge is of potassium chloride. The two large sources of potential are the metal interfaces; the relatively large area of the metal surface and the fact that the fluid surrounding it is protected from movements make for stable potentials, and the large surface covered with an excess of silver chloride makes for a relatively high degree of nonpolarizability. Repeated tests with electrodes of this sort have demonstrated that, as used in these experiments, they distort neither fast nor slow potential changes of the amplitude actually recorded from the brain, and that they do not show spontaneously fluctuating potential changes. A disadvantage is their resistance—a 40 μ electrode often has a resistance of 0.5 to 1.0 MΩ.

It is desirable to determine what potential differences occur between two points very close together in the brain. A simple way to be certain that two micro-electrodes embedded in tissue actually are a given small distance apart is to have them fastened together as a pair (see diagrams, Fig. 5). The two half-cells forming each pair have been the same as described above for single electrodes. Two pieces of capillary tubing are fastened together with de Khotinsky cement. They are partially fused together with a microflame and then pulled out to microscopic dimensions. After breaking off both at the desired length, one is chipped away as far back as is desired; this is done with a fine needle under the dissecting microscope. The dimensions of the pipets of the smallest pairs yet made have been about 25 μ; larger sizes are more easily made. The distance between the pipet tips may be any desired length; approximately 100 μ is the shortest actually used in the experiments. For testing potential gradients along any particular axis in the brain, it is desirable that the ratio, electrode separation to pipet diameter, be large; in most instances it has had a value of 3 or more. Such micro-electrode pairs may be used in any of several ways. One may record between the two micro-electrodes of such a pair on a single channel; or with two recording circuits or by successive records with one channel, one may record between each of the two micro-electrodes and a common, larger electrode ordinarily placed at a distance. It is possible to record reliably certain potentials with an electrode system consist-

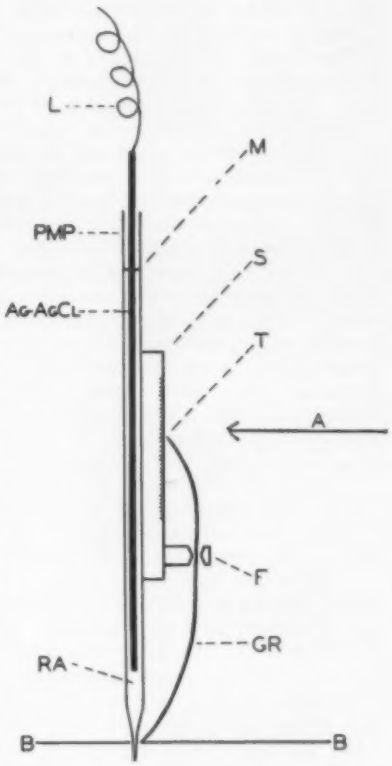


FIG. 1. Diagram of a microelectrode and a device for determining the distance it has penetrated into the brain. *BB*, the surface of a brain into which is inserted the tip of the pyrex micropipet *PMP*. *RA*, Ringer-agar filling the micropipet up to the level *M*. *Ag-AgCl*, chlorided silver wire dipping into the pipet. *L*, lead going from the silver wire to the amplifier. *S*, a graduated metal scale upon which the micropipet is mounted. [The scale is fastened to a rod (not shown) which is carried by the micromanipulator.] *GR*, a fine glass rod which passes through the hole in *F* and rests on the surface of the brain near the point of entry of the micropipet. *T*, the tip of the glass rod; its movement relative to the scale is observed with a horizontal microscope the optical axis of which is indicated by the arrow *A*.

* The micropipets have been pulled either by hand or with the machine devised and kindly loaned by Dr. L. G. Livingston, from thoroughly clean pyrex capillary tubing (outer diamter, 0.85 mm.; inner diameter 0.60 mm.).

ing of two high-resistance half-cells, but the difficulties and dangers are much greater than when one of the electrodes is of low impedance. When the amplifying system is not differential it is most important to test whether the potential changes recorded are really occurring between the two micro-electrodes and not between the one connected to the amplifier grid and a diffuse lead on the animal whose circuit to the amplifier ground is completed by capacity and leakage. Such capacitative connections are the more important the higher the frequencies recorded. Tests have proved that potential changes of the duration of the pentobarbital waves of the cortex may be recorded reliably between two micro-electrodes (see below); with much faster changes, of the duration of axon spikes, this is not true. Fortunately, the capacity between the solution and wire within a micropipet and the external conducting medium in which it is placed is not great enough to offer a shunting circuit which appreciably interferes with measurements of even rapid, axon-like potentials; the same is true of the capacity between two closely approximated members of a pair of micro-electrodes.

Effects of the grid current of the first tube of the amplifier flowing through the electrodes must be considered, for because of the very small surface area of a micro-electrode a considerable current density may exist in tissue near its tip due to the passage of only minute currents. Tests showed that the grid current from the amplifier used with the cathode ray tube (see below) flowing through a micro-electrode of tip diameter about 40 μ had no detectable effects upon axon-like spikes which were being recorded from the hippocampus; the observed activity was unaffected by the presence or absence of a 2-microfarad condenser placed in series with the electrode. Since these are the potentials which arise in the most localized way near the tips of micro-electrodes they are the ones most likely to be influenced by grid current flow.

There are two requirements for the manipulation of micro-electrodes in a controlled way; rigid fixation of the tissue (brain) relative to the electrode, and a method of moving the electrode through the tissue by small steps, as accurately measurable as possible. Satisfactory immobilization of the animal has been obtained by a three-point fixation of the skull. The jaws are fixed with a head-holder of the usual type, arranged to be as rigid as possible, and drills are forced tightly against the mastoid processes of the temporal bones. The electrodes were held and moved by means of the small Emerson micromanipulator. Ordinarily the micropipet is fastened to a small steel scale, graduated in 0.5 mm. intervals, which is carried by a brass rod firmly clamped in the manipulator. A horizontal microscope with ocular scale makes it possible to determine the movement of the electrode relative to its original position with some precision—to 100 μ or better. The movements of the electrode through the tissue are given less precisely because of the possible compression of the brain; accordingly the actual movement of an electrode relative to the brain may be somewhat more or less than its movement relative to the skull, the manipulator or other fixed points. For this reason a so-called "depth-measurer" has been devised (see Fig. 1). Movement of the electrode scale *s*, relative to a point on the ocular scale of the horizontal microscope represents the distance which the electrode has been moved vertically. Movement of the end *t* of the tiny glass rod *gr* relative to the ocular scale represents the extent to which the brain surface in the region of the penetration of the electrode has been compressed. Movement of *t* relative to the electrode scale represents the distance to which the electrode has penetrated the brain tissue. The use of such differential measurements is quite essential in investigations of the isocortex. In the case of the hippocampus it is not so necessary if the penetrating electrode is small, for the pial layers are thin and the shape of the tissue is such that it exhibits less tendency to compression.

More precise localization of micro-electrodes requires a histological examination. Fortunately the position of even a small micro-electrode may usually be located in sections because the track of the pipet stands out due to the disruption of the tissue. The histological procedure was patterned after the ones of King (1910) and Sugita (1917, 1918), devised to cause little volume change. The tissue was fixed in Bouin's fluid, cut serially in paraffin at 20 μ and stained with carbo-thionin. The end of the visible track of injury in the tissue represents the deepest point to which the tip of the electrode has been inserted (Fig. 7B and 7C) and ordinarily may be very precisely located. An intermediate point on the path of the electrode may be marked by a lateral movement of the electrode, carried out with the manipulator, and is without danger of vertical movement if the pipet is sufficiently rigid; the lesion thus produced has a shoulder which may be detected in the sections.

Three types of recording apparatus have been used. In some experiments a direct-

coupled amplifier (described by Forbes and Grass, 1937) was used with a Hindle string galvanometer. The string was generally slightly more than critically damped; the time taken for two-thirds of the total deflection to a constant current was about 6 msec. Two channels of resistance-capacity-coupled amplifiers working into oscillographs writing with pens on a paper tape (as developed by Mr. Albert M. Grass) proved useful when two simultaneous records were desired. The amplifiers could be used either with a grid-ground input connection or differentially. The frequency characteristic was linear to about 80 cycles per second, above which it tapered off gradually. Therefore it was not adequate for recording potentials of brief duration. The grid resistors of the input circuits were only 0.5 M Ω, which led to decreased efficiency in recording with high-resistance electrodes. Using the amplifiers with grid-ground connections to record on two channels between each of two micro-electrodes as grids and a common ground, the coupling between the two channels was not significant provided the resistance of the common electrode was very low. Most useful, because of its sensitivity to high frequencies, has been the cathode ray tube with the usual amplifying circuits. The grid resistor was small (0.5 M Ω). The input circuit could be used with differential or grid-ground connections; the grid-ground circuit proved satisfactory for leading with one high-resistance and one low-resistance electrode.

The difficult problem of stimulating afferent fibers in the volume of the brain only a few millimeters from the recording electrodes on or in the hippocampus was solved initially by using a thyratron-controlled condenser discharge and a balanced Bishop coil (Bishop, 1927), and later by the use of a circuit suggested by Dr. Tönnies in which thyratron-controlled condenser discharges activate the primary of the General Radio Transformer 578A, the special feature of which is electrostatic shielding between the coils; the secondary leads, shunted by 2000 Ω and each with a 0.005 μF series condenser, connect with the bipolar stimulating electrodes.

Preliminary experiments were performed on chickens of various ages. Cats and rabbits have been used in most instances, however, either under anesthesia (generally pentobarbital sodium) or decorticate. Decorticate animals were immobilized either by a thoracic spinal section and the cutting of the brachial plexi, or by cervical cord section.

The Isocortex

Observations

The experience of Derbyshire, Rempel, Forbes and Lambert (1936) suggested that pentobarbital sodium would be a most useful anesthetic for experiments designed to dissect cerebral electrical activity. These authors found that brain-activity patterns under light pentobarbital are similar to the unanesthetized, with predominant excursions of as large as several hundred microvolts occurring at frequencies of 5 to 15 per sec. As the anesthesia is deepened the waves become less frequent (5 to 10 per sec.) and there is a smaller amount of irregular, fast activity superimposed upon them. In very deep stages, waves of the same duration and amplitude may appear as isolated excursions.

Figure 2 demonstrates how greatly the cortical activity occurring under deep pentobarbital differs from the ether pattern (cf. Beecher, McDonough and Forbes, 1938). The electrical changes were recorded between an indifferent ground electrode and a micropipet (diameter 30 μ) which had been lowered about 0.5 mm. into the lateral gyrus (cat). The initial anesthetic was ether (moderately light); this was then discontinued and pentobarbital injected. Under ether rhythmic waves of rather striking regularity at over 40 per sec. characterize much of the record. With the transition to pentobarbital this frequency decreased progressively; at first there was little other change in the characteristic pattern. When the pentobarbital anesthesia be-

came deeper, the isolated excursions mentioned above became strikingly apparent. These unitary-appearing potentials, occurring during the deeper stages of pentobarbital anesthesia, are easier to investigate as isolated events than the small, rapid waves observed with ether.

Using micro-electrodes, it was found that in the deeper stages of pentobarbital the baseline becomes smooth except for occasional isolated excursions which often recur with a remarkable constancy of wave form and seem to have some similarity to the "strychnine spikes" described from the

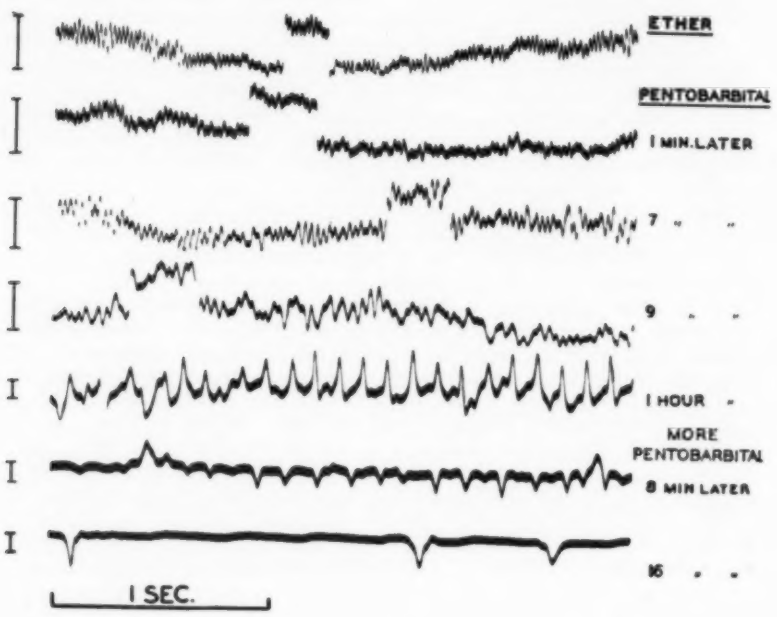


FIG. 2. Records from isocortex of cat; string galvanometer and direct-coupled amplifier. Grid, a 30 μ micropipet inserted 0.5 mm. into the anterior sigmoid gyrus; ground, a silver plate under the skull. Anesthesia as described on the figure. In each of the first four records a calibration potential of 200 μV was introduced into the circuit for a brief period. 200 μV indicated at left of each record. Upward excursion signifies grid negative. January 25, 1937.

sensory cortex by Dusser de Barenne and McCulloch (1936, 1938). Figures 2 and 4D show characteristic pentobarbital spikes in the cat and Fig. 3 demonstrates that the same phenomena occur in the pallium of the chicken. In the latter, during the deepest stages of narcosis the recurring pairs of excursions, regularly separated by a nearly constant interval, suggest a more or less stable linkage of physiological units.

As recorded in the cat the waves have durations of from 30 to over 150 msec. and may be as large as 200 or even 300 μV. These waves may present simple monophasic or diphasic forms or in some cases may be complex. Often a particular wave form may recur at intervals over a considerable period. In moderately deep stages of anesthesia the excursions often appear in series having characteristic rhythms of 5 to 7 per sec. (Fig. 4A and 4B).

The waves of a series sometimes show progressive changes in size (Fig. 4A), suggesting a systematic recruitment of elements. Occasionally the successive waves increase in duration in a way to be expected if activity in component elements were to become more dispersed temporally. Sometimes negative and positive excursions appear in alternation (Fig. 4C). These characteristics justify application of the term "units of electrical activity" to the pentobarbital excursions. It should be pointed out that the nature of the activity under deep pentobarbital may not be due to the drug *per se* but rather to secondary effects referable to its action on blood pressure and possibly other characteristics of the *milieu intérieur* (Beecher, McDonough and Forbes, 1938).

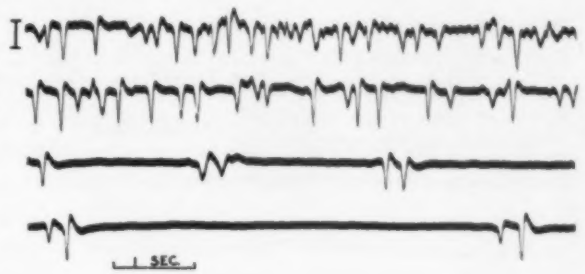


FIG. 3. Chicken 3 weeks old; successive stages of pentobarbital anesthesia as recorded with string galvanometer and direct-coupled amplifier. Micro-electrodes (tip diameters 35 and 40 μ) 1 mm. apart and pushed just through the surface of the occipital region of the pallium. 200 μV indicated at upper left. April 27, 1936.

The contours of the deflections as shown in string galvanometer records are ordinarily quite smooth, but occasionally show notches or discontinuities which indicate that a more rapid recording instrument might reveal more jagged outlines indicative of the participation of faster components in the formation of the waves. Furthermore, these excursions have been recorded not only with micro-electrodes but also with macroscopic electrodes on the surface of the brain. There is little doubt, therefore, but that the pentobarbital "units" are functional rather than anatomical and represent activity in a number of cellular elements acting together in an organized way.

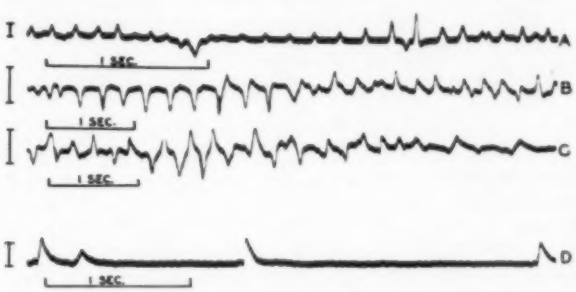
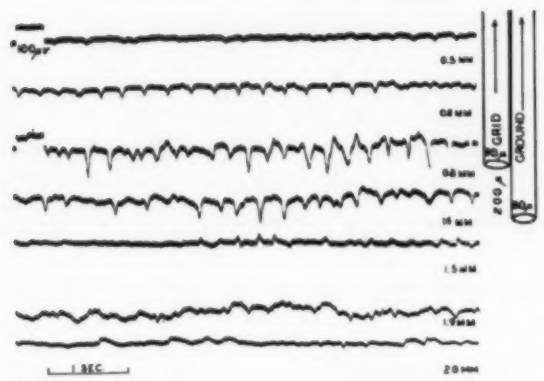


FIG. 4. Types of activity in the cerebral cortex of the cat in the deeper stages of pentobarbital anesthesia. Direct-coupled amplifier and string galvanometer. 200 μV indicated at left. Grid, a micro-electrode, diffuse ground. April 27, 1936.

On the other hand the existence of sharply localized potential gradients, in the vertical axis at least, is suggested by experiments in which the activity recorded between a micro-electrode and an indifferent ground changes greatly as the localizing electrode is pushed into the cortex by small steps. It is the use of pairs of micro-electrodes, however, which has given the most significant information about the spatial relations of activity in the cortex (cat, lateral gyrus) under pentobarbital. The results, based on records taken



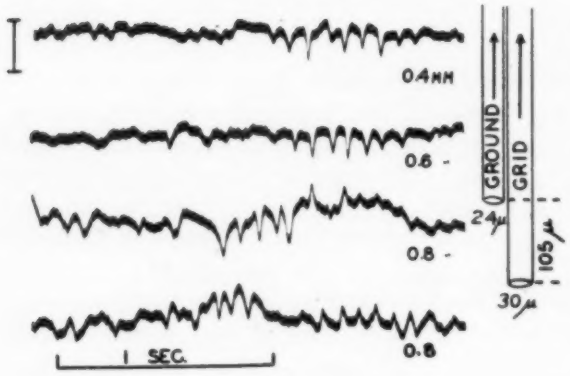


FIG. 5A. Cat under pentobarbital; records from various depths in the lateral gyrus. Electrodes—the microscopic pair shown on the figure. Direct-coupled amplifier and string galvanometer. Upward excursion signifies grid (shorter electrode) negative. Time and voltage scales as indicated. March 5, 1937.

B. Cat under pentobarbital; records from various depths in the lateral gyrus. Electrodes—the microscopic pair shown on the figure. Direct-coupled amplifier and string galvanometer. Upward excursion, grid (deeper electrode) negative. 500 μV indicated at upper left. March 17, 1937.

with the string galvanometer and the ink-writing oscillographs, may be stated as follows:

(1) Recording from a pair of micro-electrodes with a vertical separation of 105 to 300 μ (the diameter of each pipet being half or less than half of the separation distance), it is found that the activity varies with the depth to which the electrodes have been pushed into the cortex (Fig. 5A). Controls demonstrate that the electrical changes recorded really occur between the two micro-electrodes and not between one of them and a diffuse lead coupled with the amplifier ground by capacity; one could not be sure of this if the potentials were of axon-spike duration or briefer (see page 90).

(2) In the lateral gyrus maximum activity is recorded when the electrodes are at a depth of 0.5 to 1.5 mm. and are therefore in gray matter (Fig. 5A).

(3) Changes of position of the electrodes as slight as 0.1 to 0.2 mm. on the measuring scale (and probably no more in the brain) may cause a drastic change in the recorded electrical activity (Fig. 5A). This fact is proof of localized "pick-up" by this type of electrode pair.

(4) In the lateral gyrus the magnitude of the spikes as recorded from vertical micro-electrode pairs with separation distances as small as 105 μ (the smallest tested) may be as large as 200 μV (Fig. 5B); no larger po-

tentials have been obtained regularly from various other combinations of macroscopic and microscopic electrodes placed much farther apart on and in the lateral gyrus.

(5) The potentials between each electrode of a pair and a common gross electrode on the skull recorded simultaneously on two independent channels are sometimes very similar (Fig. 6, records at depths of 0.3 and 1.0 mm.). Under these conditions, the sources of potential change are taken to be at some distance from the micro-electrodes. Localized recording is not indicated.

(6) In some positions of the electrodes, however, the records of two

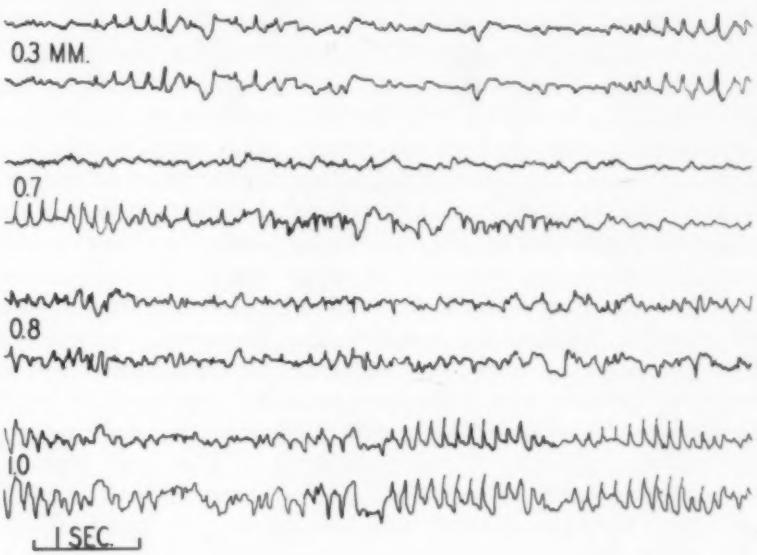


FIG. 6. Cat under pentobarbital; tracings of records from 4 positions of different depth in the lateral gyrus. Two-channel recording with capacity-coupled amplifiers and ink-writing oscillographs. Grids, the two electrodes of the pair shown in Fig. 5A. Common indifferent ground. Upward excursion signifies grid negative. Time scale shown on the figure. The largest deflections are about 200 μV. March 17, 1937.

channels are much more dissimilar than similar (Fig. 6, at depths of 0.7 and 0.8 mm.). That this condition should prevail at times is a corollary to the fact that large potential differences occur between the electrodes of a microscopic pair (statements 1 and 4). It offers proof both that steep, localized voltage gradients occur in the brain and that micro-electrodes can record these.

Discussion

The data thus verify the two predictions of the potential theory which were mentioned in the Introduction (p. 74); these are that micro-electrodes placed in some positions in active tissue should lead off large potentials and that (corollary to this) under certain conditions they should record in a localized way.

In animals under relatively light chloroform and ether anesthesia, Adrian and Matthews (1934) examined the potentials led from electrodes in various positions on the cortex and in a layer of Ringer's solution placed upon it. The faster components were recorded only by electrodes placed on or very close to the cortex itself and appeared even when the electrodes were only a millimeter or two apart; whereas the slower waves appeared only with electrodes separated by greater distances and were present even if these were raised a few millimeters away from the cortex into the Ringer's solution. Adrian and Matthews concluded that the slow waves (2 or 3 per sec.) were the summations of faster components arising from the somewhat asynchronous activity of a large number of units occupying a considerable cortical area and that the elementary building blocks of which the cortical potentials were formed probably have durations of something like 10 to 100 msec.—relatively brief but still much slower than axon spike potentials.

In the case of potentials led from two micro-electrodes extremely close together in the cortex, the possibilities for spatial summation are greatly reduced because the elements of only a relatively small volume of tissue can contribute significantly to the record. We have not examined cortices under conditions in which slow potential changes form a prominent feature of records taken with gross electrodes some distance apart. The pentobarbital deflections of 30 to 150 msec. duration, however, no doubt represent the faster type of cortical wave. It is therefore in confirmation and extension of the results of Adrian and Matthews that we find this type of activity occurring between points in the cortex separated by only a very small vertical distance.

These results suggest the value of histological preparations making possible correlations between the precise positions (*i.e.*, to distances small compared with 100 to 200 micra) occupied by micro-electrodes and the cellular structures of the region. Observations of this sort have been made, not on the isocortex, but in the hippocampus; further discussion is deferred until the results have been presented.

The Hippocampus

Observations

The hippocampus may be considered as a simplified cortex, for in each of its two principal divisions, the Ammonshorn and the Fascia dentata, one cell layer only is highly developed. Moreover its most numerous cells, the pyramids, are oriented in a relatively simple manner, with the axons, cell bodies and dendrites arranged in fairly well-defined strata. Thus, though the histological structure is of the same general sort as in the ordinary cortex, the geometrical arrangement of the cells and their processes is much less complicated. The presence of afferent pathways which may be stimulated in isolation is extremely useful, for it permits repeated and reproducible synaptic stimulation of the cells of the hippocampus. Further, the finer details of structure have been well worked out, particularly by Cajal (1911) and Lorente de Nó (1934) (see Fig. 7).

The hippocampus is a relatively long body which curves about the floor of the lateral ventricle. Sections perpendicular to the long axis in all parts, except the extreme ends, have nearly the same structure. In the cat and rabbit exposure of the hippocampus by ablation of the isocortex overlying it dorsally reveals that the surface of the part a few millimeters (3 to 8) from the midline is more or less horizontal when the animal's head is in the usual position; this is convenient for the introduction of electrodes. Further, parasagittal sections through the brain in this region are not far from normal to the long axis of the hippocampus. For these reasons this portion was chosen as the most convenient for experimentation.

1. *Injury discharge.* The insertion of an electrode into the hippocampus is often immediately followed by a more or less extensive outburst of electrical changes (Fig. 8 and 9) apparent in recordings taken between this electrode and another placed at a distance. Such activity has been designated "injury discharge" because it is transitory, gradually declining to extinction within a short time—often within 30 seconds. The peak-to-peak voltages generated during such a discharge may amount to a millivolt or more and the form may approximate a sine wave or may consist of approximately monophasic deflections of either polarity or of diphasic deflections with a faster and a slower phase. The slower components of the injury discharge are similar to the "slow waves" to be mentioned below. As recorded with a relatively large electrode (bare silver wire several tenths of a millimeter in diameter inserted for one millimeter into the hippocampus), the faster components rarely have a duration as short as 10 msec.; they are therefore much longer than the axon-like spikes described below. As recorded with a micro-electrode, however, rapid deflections of the duration of axon-spike potentials sometimes, but not always, accompany the slower deflections of an injury discharge; when present the axon-like spikes ordinarily continue long after the slower components have ceased to appear.

As would be expected from mechanical considerations, the injury discharge due to the movement of a micro-electrode in the hippocampus is probably to be related to a disturbance which is limited to a small volume of tissue. The evidence is that, although large potential differences may be recorded between a micro-electrode a millimeter or less below the surface of the hippocampus and a concentric electrode on the surface, corresponding changes on a channel recording between the surface electrode and an indifferent electrode at a distance are small or absent (Fig. 9).

The injury discharge has not been examined in great detail. It is apparent, however, that it resembles in some ways the injury effect reported from the cortex by Adrian and Matthews (1934) and which was relegated by them to the cell bodies and dendrites of gray matter because it was very different from the pattern of small, rapid potentials recorded from the deeper white matter and from nerve trunks pierced by needle electrodes. Bishop (1936) has presented injury discharges from the optic cortex which are not unlike those of the hippocampus.

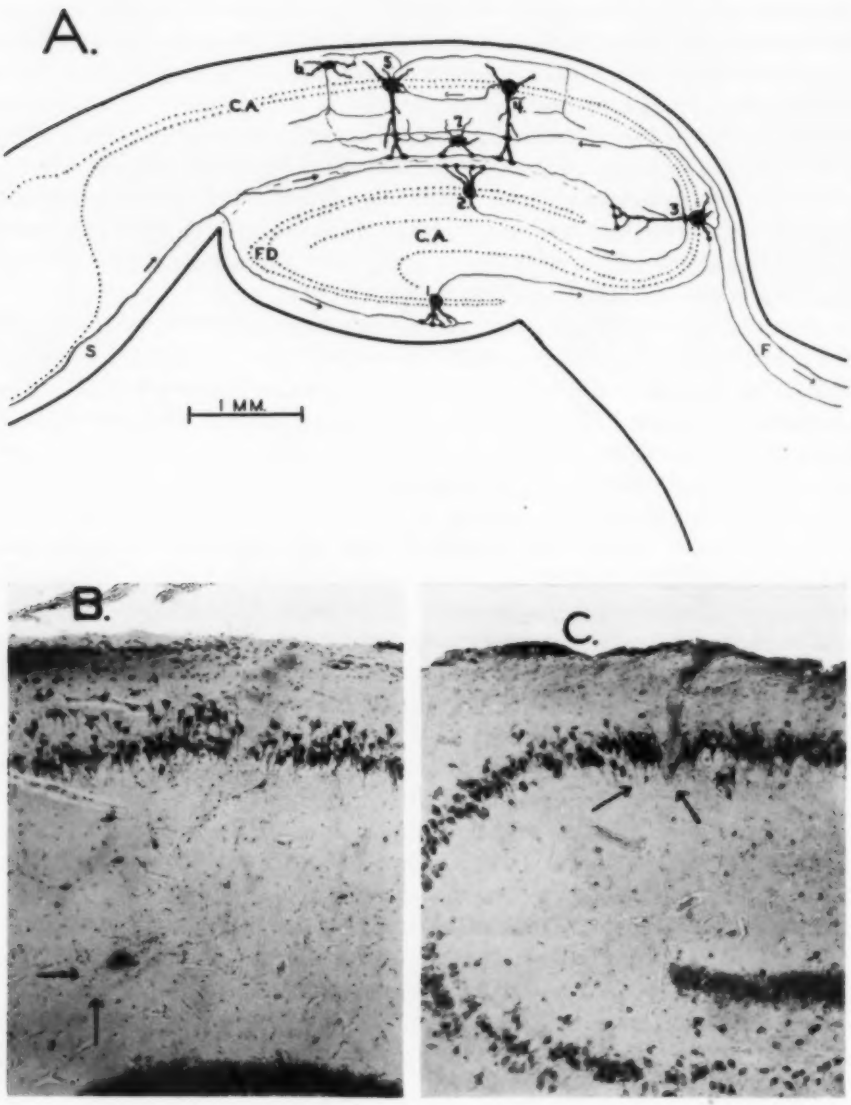


FIG. 7A. A diagrammatic parasagittal section (about 3 mm. from the midline) of the hippocampus of the rabbit. Outlines from an actual section, details from the accounts of Cajal and Lorente de Nó. (The size of the cell bodies is exaggerated.) Arrows indicate the direction of transmission of impulses. The hippocampus consists of two divisions, the Ammonshorn (CA) and the Fascia dentata (FD). The main cell type of the Ammonshorn is the pyramidal cell (3, 5); the bodies of these cells form a compact layer (the Stratum pyramidale, CA) which is conspicuous in Nissl preparations (Fig. 7 B and C). The axons of the pyramidal cells are the efferents from the hippocampus; they make up a large portion of the alveus (layer of axons on the dorsal or ventricular surface of the hippocampus) and the fimbria (F). The fimbriae of each side course cephalad and meet; as the fornices they pass ventrad and then caudad to end in the mammillary bodies. The axons of the pyramid basket cells (4) do not leave the hippocampus but form synapses on the cell

2. *Slow waves.* A conspicuous type of autonomous activity in the hippocampus as it has been investigated in experiments on cats is what we have termed the "slow wave." In general appearance the slow waves resemble the pentobarbital waves of the isocortex; there are, however, a number of differences.

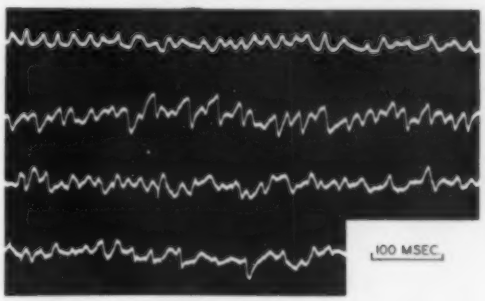


FIG. 8. Cat under pentobarbital, cathode ray. Records between an indifferent electrode and an uninsulated silver wire, a few tenths of a millimeter in diameter, pushed ca. 1 mm. into the hippocampus. Records from different positions shortly after inserting the silver wire. No axon-like spikes are seen. The larger deflections are about 100 μV. June 13, 1938.

The slow waves are recorded either from gross electrodes on the surface of the hippocampus or from micro-electrodes within it. That they are the result of activity of tissue within the hippocampus, and not due to current flow from activity originating in the subjacent thalamus or elsewhere, is indicated by several facts: they may be recorded between two micro-electrodes only a few tenths of a millimeter apart in the dorsal part of the hippocampus, two or three millimeters removed from other parts of the brain; they are recorded between a micro-electrode only 0.2 or 0.3 mm. below the surface and a concentric surface lead; as the micropipet is pushed deeper into the hippocampus the sign of the slow waves may change once or even twice; they may be recorded with as high voltage be-

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bodies of the pyramids. Some short dendrites of the pyramidal cells pass into the Stratum oriens (subjacent to the alveus), but the most conspicuous ones extend into the hippocampus for several tenths of a millimeter as the long shafts of the Stratum radiatum and arborise terminally in the Stratum moleculare. Afferent fibers (S) from the Area entorhinalis establish collateral synapses with the tips of these dendrites. The principal cell type of the Fascia dentata is the granule cell (1, 2), the bodies of which also form a compact layer (FD). Their dendrites are also in contact with the afferent fibers from the Area entorhinalis. Their axons (the mossy fibers) pass part of the way around the Ammonshorn in two tracts, one on each side of the stratum of pyramidal cell bodies, and make synapses with the dendrites of the pyramidal cells; none of them leave the hippocampus. In addition to the main cell types there are various cells with short axons, of which 6 and 7 are representative. The various regions along the long axis of the hippocampus are correlated by an axial association bundle. Correlation of the various parts of a segment (section perpendicular to the axis) is made possible by recurrent collaterals from the axons of some of the pyramidal cells and by the mossy fibers.

FIG. 7B. Photograph of a parasagittal section through the hippocampus of a cat, showing a large lesion made by inserting and moving horizontally a pair of micro-electrodes. As the movement was made forward (to the right in the picture), the position of the deeper electrode before the horizontal movement is represented by the extreme lower left portion of the lesion, indicated by arrows. Nissl stain. November 4, 1937.

FIG. 7C. Photomicrograph of a parasagittal section through the hippocampus of a cat, showing the lesion made by a pair of micro-electrodes pushed into the lower edge of the Stratum pyramidale. An adjacent section shows a small group of cells immediately below the end of the lesion. Axon-like spikes were recorded from this position. Nissl stain. December 23, 1937.

tween electrodes only a small distance (a few tenths of a millimeter) apart on or in the hippocampus as between electrodes much more widely separated but still within the hippocampus.

In some experiments potential changes of this sort may be as large as 200 or 300 μV.; in others they may be inconspicuous or essentially absent. They vary in duration from 20 to 70 msec. The successive excursions on a single record may vary considerably in both magnitude and wavelength. More than that, the wave-form may change from wave to wave, in

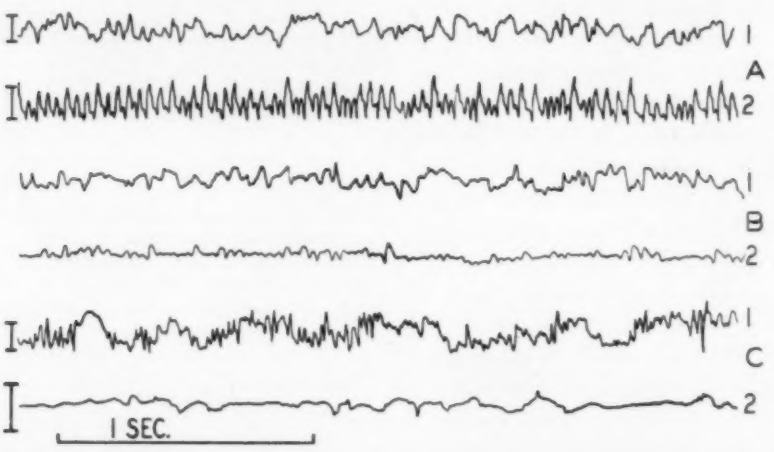

FIG. 9. Cat, hippocampus. Injury discharges recorded with ink-writing oscillographs. Pentobarbital.

A and B, December 4, 1937. Grid of channel 1 an indifferent electrode, grid of channel 2 a microelectrode of 57 μ tip diameter. Common ground for the two channels a silver ring on the surface of the hippocampus, surrounding the micropipet. A, about 20 sec. after moving the pipet from 0.9 to 1.0 mm. into the tissue (depth readings). B, 1 min. later. The baseline of channel 2 later became quite smooth. Note that the large potentials recorded on channel 2 hardly appear on channel 1 in spite of its higher amplification. Voltage calibration for channel 1, 100 μV; for channel 2, 500 μV.

C, another experiment, December 11, 1937. Grid of channel 1 a micropipet of tip diameter 40 μ. Grid of channel 2 an Ag–AgCl wire on the fimbria. Common ground a semicircular Ag–AgCl wire about the micropipet on the surface of the hippocampus. Record taken a few seconds after moving the micropipet from a depth of 0.8 to 0.9 mm. (depth readings). The activity of the upper channel rapidly decreased during the next 30 sec. Voltage calibration for both channels, 100 μV. Time as indicated.

that the peaks may be reached after variable fractions of the durations. A most striking feature of the form of the slow waves is that they are usually very nearly or quite monophasic.

In various experiments these waves have been recorded from most of the parts of the hippocampus. As recorded between a localizing electrode in the tissue and an electrically indifferent region, the sign of the slow waves may be either negative or positive with reference to the localizing electrode. It is possible to make certain generalizations relating the position of micro-elec-

trodes in the tissue to the sign of the waves as recorded. Usually during a wave the region of the Stratum pyramidale of the dorsal portion of the Ammonshorn becomes negative to the ventricular surface of the hippocampus or to a remote region. Similarly deeper regions of the hippocampus are often positive and the deepest portions (the region of the lower blade of the Fascia dentata) negative to the surface. Two-channel recording with the two micro-electrodes of a pair as grids and a surface electrode as a common ground indicates that often two points 0.2 mm., more or less, apart within the hippocampus show similar changes in potential relative to the common electrode; occasionally differences such as those obtained in the isocortex occur. This may be summarized by stating that the mechanisms responsible for the slow waves are not limited to localized regions within the hippocampus and that the volume of tissue active in the production of any particular slow wave is not highly restricted (compare axon-like spikes, described below).

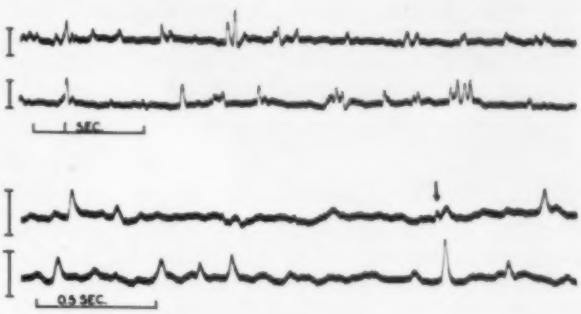

FIG. 10. Cat, hippocapmus. "Slow waves." Pentobarbital, string galvanometer and direct-coupled amplifier.

Upper records, experiment of November 5, 1937. A pair of micro-electrodes, one near surface of hippocampus, the other in the Stratum pyramidale (see Fig. 7B). Upward excursion, deep electrode negative. Voltage calibrations, 200 μV. Time as indicated.

Lower records, experiment of December 14, 1937. Grid, one of a pair of micropipets, the tip in or at the ventral edge of the Stratum pyramidale. Ground, a surface electrode. Note the group of 3 axon-like spikes (indicated by arrow); they had approximately the same amplitude as the slow waves (cathode ray visual observations) but were greatly reduced in this record because of string inertia. In this and all subsequent figures upward deflection signifies grid negative. Voltage calibrations, 200 μV. Time as indicated.

Important for the interpretation of the slow waves is that their contours, even as recorded on the cathode ray oscillograph, show no greater irregularities than are to be found on other portions of the record—no indications of the participation of fast components in their formation appear. Occasionally an axon-like spike may appear on a slow wave, but it is apparent that this is a chance occurrence and a mere superposition of one type of activity upon another; when the two are present together in a record they appear to be independent. Further, the fast spike is as often superposed on a slow wave of opposite sign as on one of the same sign as itself.

The slow waves generally disappear at depths of anesthesia insufficient to simplify greatly the activity of the isocortex (see above). Though the activity is decreased in amount as the anesthesia is deepened, so that individual waves may stand out more clearly, these do not persist in the very deep stages. In fact at no level of anesthesia do the hippocampal waves ex-

hibit the regular behavior (repeating wave-forms, rhythmic series of waves) exhibited by the pentobarbital excursions of the isocortex.

3. *Axon-like spikes.* When one of the electrodes of a recording system is small and is inserted into the hippocampus, rapid deflections often appear. The duration of their conspicuous phase is about 1 msec.; for this reason they have been designated "axon-like spikes." In favorable preparations they may appear as large as 500 μV; often they are only 150 to 200 μV. A most constant characteristic is that they are always micro-electrode negative in their predominant phase* (*i.e.*, phase of high voltage), though a smaller and much longer positive phase sometimes clearly follows the main negative one (Fig. 11 and 12). Frequently the wave-form is simple, but often there may be a hump or notch on either the rising or falling limb of the negativity. This is not always a mere chance overlapping, because what is evidently the discharge of the same unit sometimes appears repeatedly with the same compound wave-form. The occurrence of axon-like spikes ordinarily long outlasts the brief injury discharge. It is true that sometimes they cease to appear a few minutes after a micro-electrode has been moved to a new position, but in other instances they have persisted many minutes and even hours without abatement.

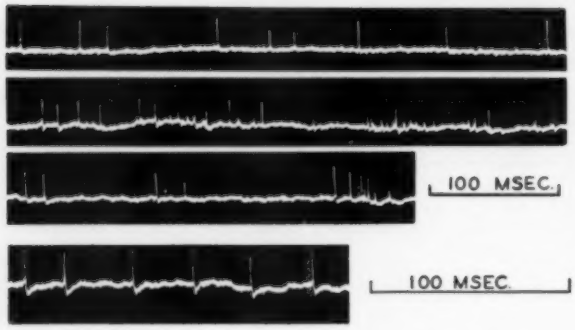

FIG. 11. Cat, hippocampus. Axon-like spikes recorded with cathode ray. Pentobarbital. March 19 and 20, 1938; June 11, 1939. Grid, a micro-electrode within the hippocampus; ground, a macroscopic indifferent lead. In this and all subsequent records upward excursion signifies grid negative. Deflections ca. 500 μV. Time as indicated.

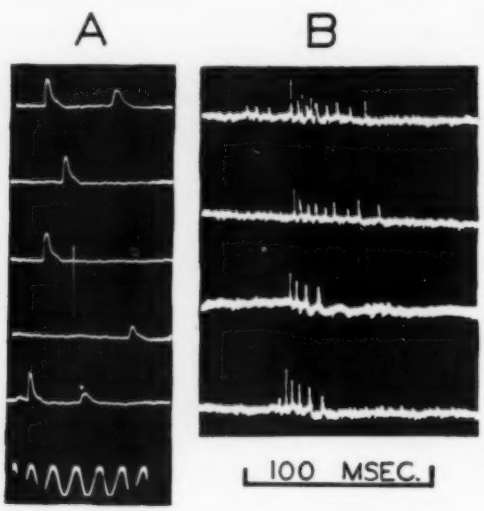

FIG. 12. Cat, hippocampus. Axon-like spikes recorded with cathode ray. Pentobarbital. Electrodes as in Fig. 11. A. High speed record, 500-cycle line at bottom. June 11, 1939. B. Groups of spikes. Time as indicated. March 19, 1938.

Perhaps the most striking feature of these axon-like spikes is

* This applies to all spikes of sufficient voltage to rise clearly out of the baseline made noisy by the high resistance of the micro-electrode. Smaller positive spikes may, and we suspect must, occur.

their frequent grouping in a declining series. It is true that often the spikes appear singly (Fig. 11), and periods of considerable activity may alternate with periods of almost complete inactivity (duration, 30 to 60 sec.). Frequently, however, the spikes come in closely grouped clusters of from 2 to 10 (Fig. 12B). Sometimes the size and spacing of the members of a group appear irregular; examination of the records then suggests that two or more units are involved. In other instances the successive members of a group exhibit a strikingly regular sequence in which they vary progressively and consistently in three ways; they decrease in size, they increase in duration, and the intervals between them increase. The changes in these three characteristics run parallel courses.

A large amount of evidence indicates that the axon-like spikes involve very localized volumes within the hippocampus. They have never been recorded with gross electrodes on or in the hippocampus, but only from micro-electrodes and then only when these were placed in certain, not all, positions within the hippocampus. Even when they appear as large as several hundred microvolts, as recorded between a micro-electrode in the tissue and a concentric surface electrode, they do not appear at all in records taken between the concentric surface electrode and another superficial one placed at a distance. When records are taken of the activity between any surface or distant electrode and a micro-electrode which is lowered into the hippocampus by small steps of about 0.1 mm., axon-like spikes appear in only one or a few vertically localized regions. In several instances paired micro-electrodes with a separation of about 200 μ were lowered into the hippocampus and records taken with these used alternately as grids and a surface electrode as ground. Axon-like spikes appeared first with the lower micro-electrode, then after the pair had been pushed in a little farther they were recorded from the upper electrode, but in no instance from both at a single position. When a micro-electrode which is in a position to record axon-like spikes is pushed in the tissue to a deeper level and then withdrawn to the original locus, the spikes have then never been recorded. It may be concluded that the tissue active in the production of the axon-like spikes is very restricted in volume, that it lies close to the micro-electrode recording the spikes, and that the potential gradients set up by its activity fall off rapidly with distance.

Over 20 depth analyses, most of them controlled histologically, were made to determine where in the hippocampus the axon-like spikes appear. The evidence of these, supplemented by many other observations incidental to other experiments, indicates that the vertically localized regions are within or at least very close to, the Stratum pyramidale—the layer where the cell bodies of the pyramids of the Ammonshorn are located. In a number of instances the spikes have been recorded when the micro-electrode was within the hilus of the Fascia dentata, in Lorente de Nó's area of modified Ammonshorn pyramids, CA4; in other experiments the micro-electrode was within, or in some instances perhaps just ventral to, the Stratum pyramidale

of the dorsal portion of the Ammonshorn. Figure 7C shows the lesion made by a micro-electrode in an experiment in which the axon-like spikes were being recorded. In only two cases do the data indicate that the axon-like spikes appeared when the micro-electrode was in another part of the hippocampus; in one of these the sections showed a lesion well within the Stratum radiatum, in the other the lesion extended to within a very small distance (50 μ or less) of the tip of the upper blade of the Fascia dentata, another region of closely packed cell bodies. It is a question whether these exceptions are valid or whether they involve errors in the histological controls.

The systematic investigation of the axon-like spikes was made in the anesthetized cat; they also occur in the decorticate and unanesthetized rabbit. The relation of the axon-like spikes to slow waves was described above; their relation to responses to stimulation will be mentioned below.

4. *Responses of the hippocampus to stimulation of its afferent fibers.* Stimulating electrodes have been inserted into the entorhinal area in order to stimulate the fibers running to the hippocampus (*S*, Fig. 7A). Ordinarily, one recording electrode was placed on the hippocampus and another put at some other position on or near the brain. Up to several per second, the frequency of stimulation had little effect on the response; stimuli were given therefore at rates of 1 to 3 per sec.

Typical responses taken from several experiments are shown in Fig. 13, A–R. In each of the experiments, activity was detected over a large area of the surface of the hippocampus; the details but not the general aspects of the responses varied with the position of the hippocampal electrode. The position of the other recording electrode was shown to be immaterial. In both cats and rabbits the first phase of a response typically represents positivity at the surface of the hippocampus. It has a latency of about 1 to 4 msec. and a duration of 6 to 20 or more msec.; it may be as large as 1 mV. in the cat under pentobarbital and much larger in the decorticate rabbit. With weaker stimuli the positive phase is generally simple; as the stimuli are increased in strength the responses become larger and sometimes more complex; often they assume a dicrotic shape. The positive phases of the responses to a slow series of uniform stimuli are very constant. The positive phase is often followed by a negative deflection which exhibits considerable variation in its size and duration, even within a single series of uniform stimuli. In some experiments it is not detectable; in other instances it is larger than the positive phase. Its duration, measured from the stimulus, varies from 40 to over 100 msec.

We have not yet obtained records from the fimbria or fornix which we can definitely state represent activity in the axons of this efferent pathway, uncontaminated by the response of the hippocampus itself. Figure 13J demonstrates that the fibers of the fimbria were conducting, however. In this case the stimulating electrodes were on the fimbria several mm. from the hippocampus and the active recording electrode was on the anterior part of the hippocampus. The di- or perhaps triphasically recorded spike repre-

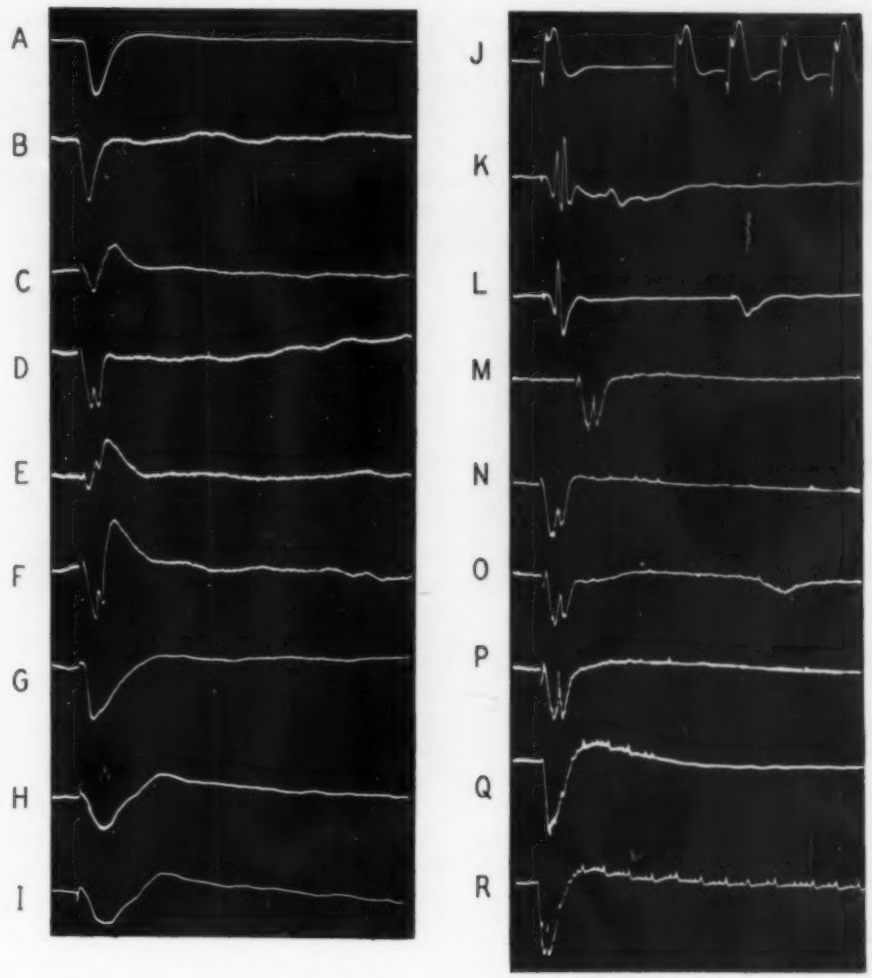

FIG. 13. Hippocampus. A–F, J, M–P, cats under pentobarbital; G–I, K, L, Q, R, decorticate rabbits. A–I, various forms of the responses to stimulation of afferent fibers from Area entorhinalis. Grid, a macroscopic electrode on the hippocampus; ground, an indifferent electrode. I, same as H, but apparatus with a much longer time constant than usual to demonstrate that the negative phase is little distorted with the customary coupling. J, stimulation of the fimbria; monopolar recording from the anterior border of the surface of the hippocampus. K–L, same as A–I, showing spike complication. M–P, responses to stimulation of Area entorhinalis, recorded from micro-electrode in hilus of Fascia dentata. Q, R, responses recorded from a micro-electrode (30 μ tip diamter) inserted into the Area entorhinalis close to the stimulating electrodes. Duration of each record, 155 msec.

senting impulses in the axons of the fimbria is followed by the larger and slower potential changes referable to activity in the hippocampus. Responses of the hippocampus to stimulation of the entorhinal area are often complicated by what is apparently the superposition of one or two spikes on the positive potential (Fig. 13, K, L; see also Fig. 16B). These are often large

and have a duration of 2 to 3 msec. They are recorded both from surface leads and from electrodes placed within the hippocampus. What may be the components of such spikes have been recorded on a few occasions from micro-electrodes placed within the hippocampus. Fig. 13, M–P shows four responses taken from a micro-electrode placed within the hilus of the Fascia dentata. The response in this position happened to have been recorded with much the same form as the surface response, but during the period of the positive potential there appeared very rapid spikes which vary con-

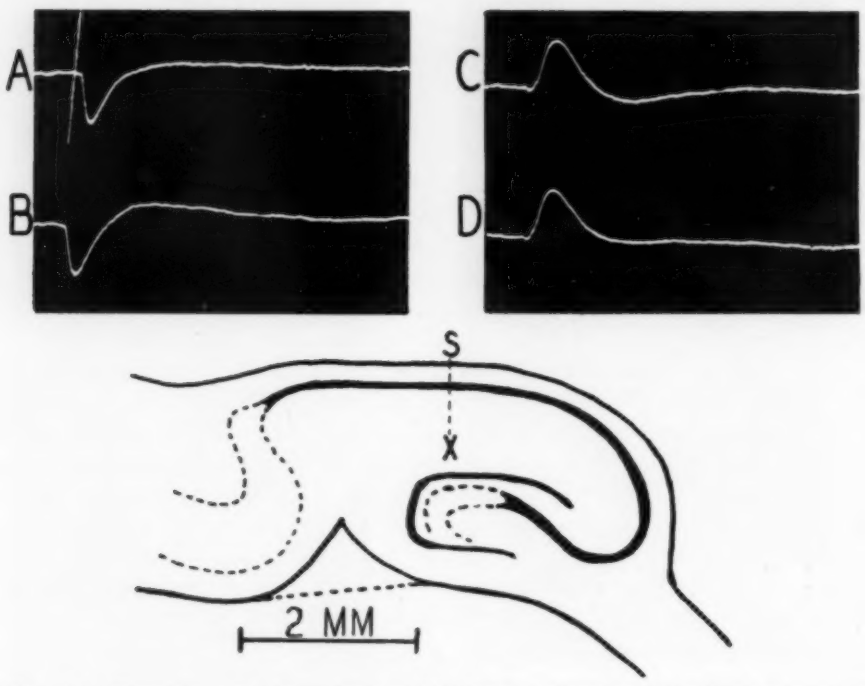

FIG. 14. Hippocampus, decorticate rabbit. Monopolar recording from micropipet of *ca.* 30 μ tip diameter. Stimulation of Area entorhinalis. A, B, micro-electrode on surface at point S. C, D, micro-electrode inserted into position *X*. Duration of each record, 120 msec. Responses *ca.* 700 μV. Simultaneous ordinates shown on *A*. Diagram traced from a section showing the lesion made by the micro-electrode. July 29, 1938.

siderably from record to record. Their principal phase seems to be negative —opposite in sign to the response on which they appear to be superposed. Spontaneous axon-like spikes were recorded from this position of the micro-electrode. Fig. 13 Q, R shows some responses taken with a micro-electrode inserted not into the hippocampus but into the entorhinal area near the stimulating electrodes. In this experiment spikes were superposed on the responses and continued in some cases as a short after-discharge; a series of stimuli at *circa* 50 per sec. was followed by an after-discharge of these rapid spikes lasting several seconds and superposed during its earlier part on slow deflections of the baseline. These spike complications do not appear in all

experiments, and when present they seem to be superposed on the slower and universally present components of the response. Consequently our analysis has been concerned only with the slower components and the interpretation of the spikes must await further experimentation.

Micro-electrodes have been used to examine the distribution of the potential changes which occur within the hippocampus during the responses to stimulation. Significant reversals of sign of electric response appear at certain depths. The rabbit has proved much more satisfactory than the cat for this purpose, both because the voltage of the responses is greater and because the hippocampus and related structures have a more favorable topography, being flatter and more spread out. Not all of the experiments are readily subject to analysis, but when certain conditions prevail it is possible, in our opinion, to relegate the greater part of the recorded responses to the perikaraya of the pyramids of the Ammonshorn. These conditions are (1) that the responses as recorded from the surface of the hippocampus be approximately the same over a large area, on the central portion of which the micro-electrode is placed and inserted; (2) that the responses as recorded from this area be relatively large compared to potentials recorded from other regions such as the Area entorhinalis in the vicinity of the stimulating electrodes; and (3) that the major potential gradients set up within the hippocampus occur in the dorsal portion of the Ammonshorn and not in the deeper parts of the hippocampus.

That the third condition is not always true is demonstrated by the records of Fig. 15A, which represent the responses as recorded between an electrode on the surface of the hippocampus and a micro-electrode inserted to various depths within the tissue. At the end of the experiment the electrode at a depth of 1.5 mm. was moved laterally to produce a shoulder on the lesion; the sections demonstrated that this position corresponded to about the level to which the tips of the dendrites of the pyramids of the upper portion of the Ammonshorn extended ventrally. It is seen that a conspicuous change in the recorded activity occurred below this level. In other instances records taken between a micro-electrode and a remote, indifferent electrode demonstrate that the response with the micro-electrode in the deeper portions of the upper part of the Ammonshorn is roughly of the same wave-form and voltage as the surface response but of opposite sign, and conspicuous changes in potential do not occur at deeper levels (Fig. 14, 15B, and 16A). In experiments giving this result, records taken at a number of intermediate vertical positions demonstrate that the level at which the sign of the response reverses differs according to what portion of the response is examined; but it is also apparent that this band corresponds to the more ventral regions of the Stratum radiatum, and not to the region of the Stratum pyramidale. Fig. 15B shows an experiment in which the response appears to consist of at least two components which reverse at somewhat different depths; in most experiments such a clear separation of components in the response does not occur.

Fig. 16B shows records from one experiment in which the responses were most significantly atypical. That the excitation of the hippocampus was different from the usual is indicated by the facts that the latency of the

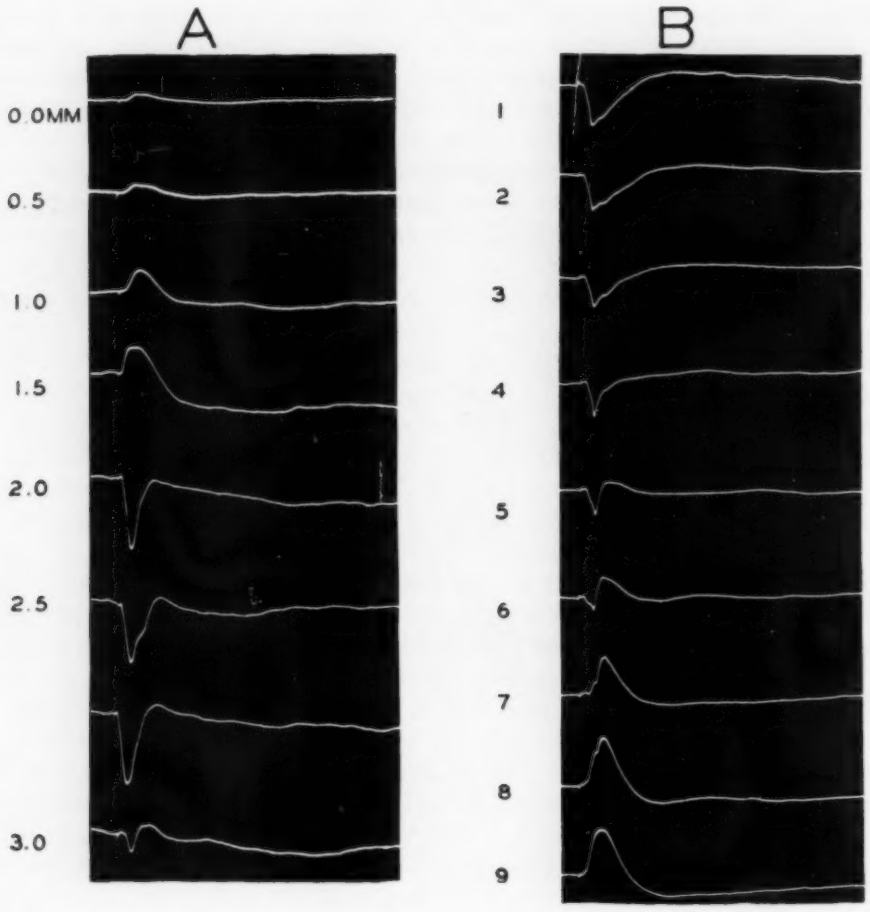

FIG. 15. Hippocampus, decorticate rabbit. Stimulation of Area entorhinalis. Duration of each record about 130 msec.

A. Recording from a micro-electrode at various depths in the hippocampus and an adjacent surface electrode. Slides showed that the depth reading of 1.5 mm. was at about the ventral border of the dorsal portion of the Ammonshorn. Sept. 23, 1938.

B. Monopolar recording from a micropipet at various depths in the hippocampus, from the surface (1) to a depth reading 1.3 mm. (9), which is about the ventral border of the dorsal portion of the Ammonshorn. Simultaneous ordinates shown on (1). August 8, 1938.

principal response (which was very large—*ca.* 7 mV), instead of being one or a few milliseconds, was about 20 msec. and that the sign of the response was surface negative in contrast to the typical surface positive. The micro-electrode was inserted, and it was discovered that the sign of the response

reversed at the shallow depth of 0.3 to 0.5 mm., in the region of the cell bodies and proximal portions of the dendrites of the pyramids. It should be emphasized that although the results obtained in this experiment were exceptional, a large number of records over a considerable period of time established their constancy for this particular preparation.

Discussion

A sharp distinction may be drawn between the axon-like spikes and the slow waves. Although we do not know what the slow waves signify in terms of the activity of neurons, no evidence has yet appeared to indicate that the latter are the summation products of numbers of axon-like spikes or other rapid components. No intermediate forms are seen. Though they have 10 to 100 times the duration of the spikes, the slow waves have contours which are smooth to the limit of resolution of the recording system. When the two types of activity occur together in the same record they show no interdependence; neither seems affected by the other even when, as occasionally happens, they are superposed. One type of activity may occur without the other. Slow waves may be recorded from macroscopic electrodes on the surface of the hippocampus; axon-like spikes never have been, in spite of their equally great or even greater voltage as recorded with micro-electrodes. Recording between an electrode on the surface of the hippocampus and a micro-electrode within it, the slow waves may be detected when the micro-electrode is in almost any position and the deflections may be either micro-electrode negative or positive; the axon-like activity, on the other hand, is obtained only from certain restricted portions of the hippocampus and is always micro-electrode negative in its conspicuous phase.

What are the axon-like spikes? Their characteristics as enumerated above—in particular, their brief duration, the restricted volume of tissue from which they are recorded, and their systematic behavior in the groups which appear in some experiments—suggest that each must represent activity in a single neuron or in a small group activated by a common agent (compare motor unit of Sherrington). It is possible that they are injury effects (cf. Adrian, 1930) due to the presence of the micro-electrode which is instrumental in recording them—since they are only recorded from micro-electrodes placed within the hippocampus, this point cannot be settled directly. A propagated disturbance arising in a portion of a neuron near the micro-electrode and traveling away to more remote portions of the same cell would account for the prominent initial negative phase and the smaller but more prolonged positivity which is seen to follow in some records (Fig. 11). On the other hand, the spikes are apparently associated with the Stratum pyramidale. In some instances spike-like activity follows the stimulation of afferent tracts to the hippocampus, appearing either superposed on the responses or as an after-discharge (Fig. 13, K–R). Furthermore, the occurrence of the spikes in groups (Fig. 12B) parallels the activity, shown not to

be an injury discharge, recorded by Adrian and Moruzzi (1939) in the fibers of the pyramidal tracts from the cortex. This evidence is not of a conclusive nature, but it leaves open the possibility that the axon-like spikes represent physiological activity in which the bodies of pyramidal cells play an essential rôle. The simultaneous decline of voltage, brevity and frequency in the groups of axon-like spikes suggests the development of fatigue or subnormality in units rhythmically or continuously stimulated, and consequent cessation of discharge.

The examination of the axon-like spikes, and particularly the results of experiments with two micro-electrodes close together in the hippocampus, has afforded convincing evidence of (1) the localized recording which micro-electrodes permit and (2) the restricted volumes within which the electrical changes due to activity in single units are large enough to be recorded readily.

Generally during the first part of the responses of the hippocampus to the electrical stimulation of afferent fibers the surface is positive to the deeper parts of the dorsal portion of the Ammonshorn; but in the exceptional experiment (Fig. 16B) the reverse is true. Thus the difference in potential which arises across this cell layer due to activity of the hippocampus may be either of one sign or the other.

If we may interpret voltage changes such as we have recorded in terms of potential differences originating at the surfaces of neurons, then as was pointed out above, there is required the production of a greater change in potential in some portions of the active neurons than in other parts of the same cells.* The diphasic recording of axon spike potentials in nerve trunks fulfills this condition. But the length of the region active during the passage of a propagated disturbance in nerve fibers is great compared to the entire extent of many cells of short axon; it is as much as several centimeters in mammalian A fibers (Gasser and Grundfest, 1936) and a few millimeters in mammalian C fibers (Grundfest and Gasser, 1938). Consequently, though little is known of the nature of processes occurring in perikarya, it is likely that the production of the large potential differences in the gray matter is due less to the movement of a region of altered potential in the neuron than to the approximately simultaneous production of different changes of potential in adjacent regions (cf. Hughes and Gasser, 1934; Adrian, 1936). For each point on the surface of a neuron a curve might hypothetically be constructed to relate potential change with time. If during activity of the neuron discrepancies exist between the curves for the points on one portion of the neuron and for those on another, then current must flow from the one portion to the other in the medium bathing the cell. Con-

* Because in the present work only transient changes of a duration of no more than 200 msec. are considered, it makes little difference whether the entire surface of the inactive but living neuron is isopotential (as is commonly assumed) or not (cf. Buchthal, 1937, for the case of the region of the motor end-plate); we are concerned only with changes from the resting state.

ceivably such differences may exist between the axon and the perikaryon as a whole, or between different parts of the perikaryon. When present they may properly be called cell potentials and be measured in terms of the voltage drops (cf. Dusser de Barenne and McCulloch, 1939) occurring in the medium bathing the cells.

In some experiments at least the greater part of each of the responses recorded from the surface of the hippocampus and from within the Ammonshorn must be relegated to elements oriented vertically in the Ammonshorn. The potential changes are large (1 to several millivolts in the rabbit). They appear simultaneously and of approximately the same voltage and wave form over a considerable area of the surface. On the other hand, marked changes occur in the vertical direction, and these (in some experiments) are more pronounced in the Ammonshorn than in deeper regions of the hippocampus. The responses within the deeper portions of the (dorsal blade of the) Ammonshorn are reversed in sign but of approximately the same voltage and wave form as the surface response. Furthermore, the changes in the vertical axis are apparently not mirrored in any particular horizontal plane or planes, as might be expected if they arose in horizontally oriented elements. In the Ammonshorn there are, of course, many axons coursing in various directions, particularly in horizontal planes. Cells of short axon are also present in the Strata oriens, radiatum and moleculare, but in very restricted numbers (Fig. 7B and C). The pyramidal cells, however, comprise by far the most numerous cellular elements of this region, and they are vertically oriented. Consequently we infer that the greater part of each of the recorded responses is due to activity of the pyramids.

Just how closely the recorded responses parallel the sequence of changes in a single neuron is not clear. Probably the pyramidal cells are not activated merely by a single volley delivered to the synapses at the tips of the dendrites by fibers from the Area entorhinalis. The stimulus may activate the Area entorhinalis to a more or less extended discharge. Though prolonged reverberation within the hippocampus is not likely, delayed impulses presumably reach the Ammonshorn pyramids from the axons of the granule cells of the Fascia dentata, from the occasional cells of short axon in the Ammonshorn, and from the powerful recurrent collaterals of the pyramids themselves. Furthermore, there is the possibility of reverberation in longer circuits involving both entorhinal areas and both hippocampi (cf. Cajal, 1911; Lorente de Nó, 1934). Unfortunately also the character of the discharge from the pyramidal cells—the activity in the axons of the fimbria—is not yet clearly understood. Figure 13J shows that these axons could be excited directly in our experiments. It also shows that when this is done the spike potentials of even a big synchronous volley are somewhat dwarfed by the large responses apparently arising in the Ammonshorn. The spikes are recorded di- or tri-phasically, so that the voltage change to be recorded as a result of even a considerable amount of somewhat dispersed activity must be small. The short length of the axons and their relation to the hippo-

campus with its large responses has prevented us from determining with certainty what efferent impulses are associated with activity in the Ammonshorn. The "spike complex" shown in Fig. 13, K, L, and Fig. 16B may be associated with efferent discharge. However that may be, the general form of the typical response recorded from the hippocampus conforms to a pat-

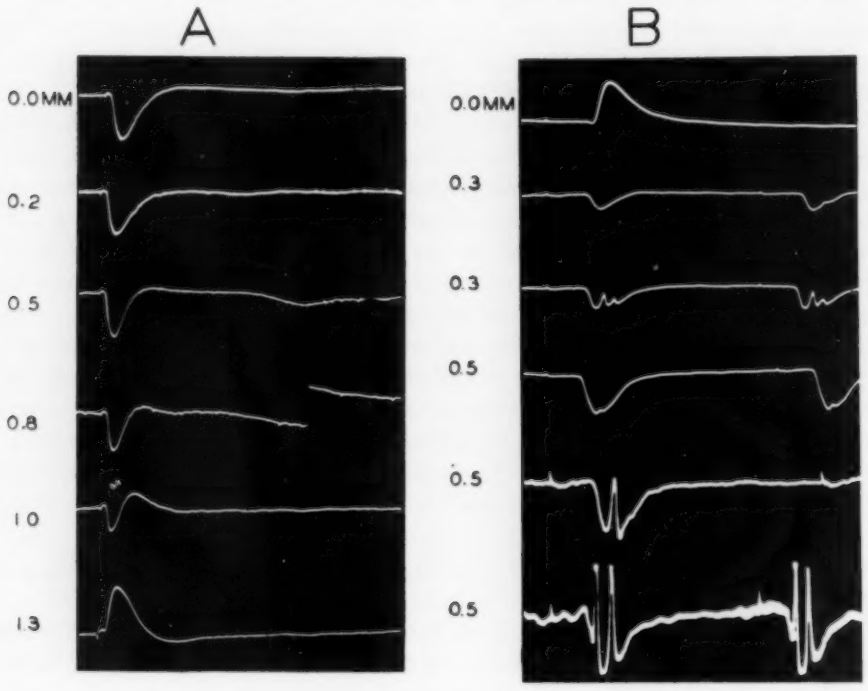

FIG. 16. Hippocampus, decorticate rabbit. Stimulation of Area entorhinalis. Monopolar recording from micropipet (tip diameter *ca.* 25 μ) at various depths in the hippocampus.

A. A typical experiment; a surface-positive response of short latency, reversing in sign in the deeper parts of the dorsal portion of the Ammonshorn. Responses about 1 mV. Calibration on record at 0.8 mm. depth reading. Duration of records, 155 msec. Simultaneous ordinates as in Fig. 15 B. August 8, 1938.

B. The atypical experiment; a surface negative response of long latency, reversing in sign at 0.3 to 0.5 mm. depth reading, at about the level of the Stratum pyramidale. The facilitating effect of a fairly rapid series of stimuli was required to obtain these large responses (7 mV). The last two records were obtained later than the previous ones and with stronger stimuli. Duration of records, 160 msec. September, 22, 1938.

tern found in other parts of the nervous system (cf. Erlanger and Gasser, 1937; Eccles, 1936; Granit, 1933; Gasser and Graham, 1933).

Typically the response of the hippocampus when uncomplicated by spikes is surface positive and reverses in sign as a micro-electrode is pushed into the deeper portions of the Ammonshorn, so that the zone of reversal of potential—the vertical region whose potential is equal to that of an indifferent point—lies in the region of the deeper portions of the long spike

dendrites of the pyramids. In so far as the potential differences are due to the pyramidal cells, this signifies that the majority of active neurons have become relatively depolarized below this level—at their dendrite tips—and less so (if at all) in their more dorsal regions. It is true that activity in all units in the remainder of the hippocampus and even in more distant regions contributes, theoretically at least, to the potential changes occurring at points within the Ammonshorn. The large size and the rapidity of reversal of the potential changes in the Ammonshorn makes it fairly certain that such effects are not great enough to alter the conclusion that the pyramidal cells become, due to their own activity, relatively negative in the deepest portions of the dendrites and positive (relative to a point whose potential does not alter) in the cell body and the proximal portions of the spike dendrites.

Any complicating effect due to activity elsewhere must be even less in the atypical experiment (Fig. 16B), for not only were the potentials extremely large but the reversal of sign of the response occurred at a much shallower level, further from other elements outside the Ammonshorn. In this experiment the dorsal portions of the majority of active pyramids became negative to the deeper parts. The precise level of reversal was not clear, but it was certainly close to the cell body, so that in the majority of active cells the region of the axon and the dendrites in the Stratum oriens must have been negative relative to the spike dendrite extending ventrally.

Adrian (1936, 1938) has recorded potential changes from the surface of the electrically stimulated cortex, which resemble the hippocampal responses. He showed that, in some cases at least, the potential changes which he termed the "deep response" are due to activity in the vertically oriented pyramidal cells. In these responses the surface of the cortex became electrically positive to an indifferent region. He interpreted this as indicating that the deeper portions (part of the cell body) of the pyramids become more depolarized than the apical dendrites which extend toward the surface of the cortex. Since the orientation of the cells of the Ammonshorn is the reverse of that in the cortex, the distribution of potentials in our atypical experiment is similar to that found in the isocortex by Adrian. Dusser de Barenne and McCulloch (1938) have examined cortical activity by the use of a combination of three analytical methods—strychnine poisoning, thermo-coagulation, and depth analysis with micro-electrodes. They found that the "strychnine spike" may be analyzed into an earlier component represented by negativity (relative to an indifferent electrode) in the deeper layers of the cortex and positivity at the surface; a later component whose sign is the reverse of the first; and a final phase which is presumably similar to the first. It seems that a possible explanation of these results would be in terms either of a shifting locus of relative negativity in a single group of vertically oriented elements (pyramids) or, more probably, of the development of a locus (which shifts less pronouncedly) in each of two groups of elements. Lorente de Nó (1939) has recently obtained evidence that after

conduction of an impulse initiated by either synaptic or antidromic stimulation, the somata of the active motoneurons of the oculomotor nucleus remain negative in relation to the proximal parts of the axons for 25–30 msec. or even longer. Attention may also be called to the potential changes of longer duration which have been recorded from sympathetic ganglia by Eccles (1936), Rosenblueth and Simeone (1938) and Lloyd (1939).

To carry the analysis of the results on the hippocampus one step further, two facts may be noted. (1) Excitation in both the typical experiments (Fig. 14, 15B, 16A) and in the atypical experiment (Fig. 16B) was synaptic; that is, the cells whose activity was being recorded were stimulated not directly by the electric shock, but indirectly by activity in nerve fibers. (2) The pyramids comprise the greater part of the Ammonshorn and they are of one kind; that is, the various kinds of afferent fiber (axons from the two entorhinal areas, from the contralateral hippocampus, from the Fascia dentata, recurrent collaterals, etc.) do not each end on a certain group of pyramids. Rather each pyramid has synaptic connections with most or all of the different kinds of afferent fibers, and the synapses of each type of fiber are located on a particular region of the pyramidal cell (cf. Lorente de Nó, 1934). These facts, together with the large size of the responses, leave no reason to believe that one type of pyramid was stimulated in the atypical experiments and another in the typical. The difference between the two groups of experiments is more probably due to the synaptic stimulation of the same type of pyramid, but by fibers whose number, origin (=position of ending on the pyramids) and sequence of activation differ in the two cases. If the position of the active synapses determines the electric field set up by the pyramidal cells, the existence of a process more or less localized to the region of an active synapse and characterized by an electrical sign is indicated; and in so far as the recorded potentials are related to excitability, the effect which an impulse in an afferent fiber may have on the pyramid will be determined not only by the temporal but also by the spatial pattern of previously arriving impulses.

Summary

1. Micro-electrode techniques suitable for examination of electrical changes occurring in the nervous system are described. A satisfactory type of micro-electrode has proved to be a micropipet, filled with Ringer-agar, into which dips a chlorided silver wire. Its limitations are discussed.

2. The following two predictions have been verified by experiments on the isocortex and on the hippocampus: (a) large potential differences should be recorded from micro-electrodes at certain positions in active tissue (*i.e.*, from positions close to active elements); (b) under certain conditions micro-electrodes should record predominantly the activity of only a small volume of tissue immediately adjacent to them.

3. Deep pentobarbital anesthesia greatly simplifies the electrical patterns recorded from the isocortex (cat). Only isolated voltage changes of

considerable size and duration appear; their characteristics are such that they must be due to organized activity in groups of related neurons.

4. Further experiments have been performed on the hippocampus (cat; rabbit) because of its relatively simple structure. In addition to changes manifestly due to stimulation by injury three types of activity may be described.

5. "Slow waves" appear as spontaneous excursions of 20–70 msec. duration. They are recorded from the surface of the hippocampus as well as from micro-electrodes placed at various positions within it. Their nature is obscure but there is no evidence that they are composed of overlapping spike-like components.

6. Rapid deflections of about one millisecond in duration and always negative in their predominant phase are recorded only from micro-electrodes placed in or very near the strata containing the cell bodies of the pyramidal cells of the Ammonshorn.

7. The stimulation of the afferent fibers going to the hippocampus from the Area entorhinalis results in responses which may be recorded from the surface of the hippocampus and from points within it. The surface response ordinarily appears after a latency of one or a few milliseconds and is characterized by an initial surface positive phase (10–20 msec.) followed by a smaller, longer and more variable surface negative phase. The responses recorded from micro-electrodes inserted within the hippocampus appear of opposite sign when the micro-electrode (monopolar lead) is in the deeper parts of the Ammonshorn. In a significant atypical experiment the response was surface negative and reversed at a shallower depth in the hippocampus. The results have been analyzed in terms of potential theory and the membrane hypothesis. It is concluded that the responses to stimulation are due largely to potential changes characterizing the activity of the perikarya of the pyramidal cells of the Ammonshorn.

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